Evaluation of antidiabetic potential of whole plant extracts (ethanol and aqueous) of *Coldenia procumbens Linn.* in rats

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The Tamil Nadu Dr. M.G.R. Medical University, Chennai-32

In partial fulfillment for the award of the degree of

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Submitted by
REG.NO.261325216

Under the Guidance of
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EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"Evaluation of antidiabetic potential of whole plant extracts (ethanol and aqueous) of *Coldenia procumbens Linn.* in rats"** submitted by the student bearing **Reg. No: 261325216** to **"The Tamil Nadu Dr. M.G.R. Medical University"**, Chennai, in partial fulfillment for the award of Degree of **Master of Pharmacy** in **Pharmacology** was evaluated by us during the examination held on……

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This is to certify that the dissertation **"Evaluation of antidiabetic potential of whole plant extracts (ethanol and aqueous) of *Coldenia procumbens Linn.* in rats"** is a bonafide work done by **Reg. No. 261325216, J.K.K.Nattraja College of Pharmacy**, in partial fulfillment of the University rules and regulations for award of **Master of Pharmacy** in **Pharmacology** under my guidance and supervision during the academic year 2015-2016.

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This is to certify that the work embodied in this dissertation entitled **"Evaluation of antidiabetic potential of whole plant extracts (ethanol and aqueous) of *Coldenia procumbens Linn.* in rats" ,** submitted to "**The Tamil Nadu Dr. M.G.R. Medical University**", Chennai, in partial fulfillment to the requirement for the award of Degree of **Master of Pharmacy** in **Pharmacology**, is a bonafide work carried out by **Reg. No. 261325216,** during the academic year 2015 - 2016, under the guidance and supervision of **Dr. R. SHANMUGA SUNDARAM, M.Pharm, Ph.D.,** Vice Principal and Head, Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

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DECLARATION

I do hereby declared that the dissertation **"Evaluation of antidiabetic potential of whole plant extracts (ethanol and aqueous) of *Coldenia procumbens Linn.* in rats"** submitted to "**The Tamil Nadu Dr. M.G.R Medical University"**, Chennai, for the partial fulfillment of the degree of **Master of Pharmacy** in **Pharmacology.** It is a bonafide research work has been carried out by me during the academic year 2015-2016, under the guidance and supervision of **Dr. R. SHANMUGA SUNDARAM, M.Pharm, Ph.D.,** Vice Principal and Head, Department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

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1. INTRODUCTION

Herbal drugs

Natural products from plant, animal and minerals have been the basis of the treatment of human disease. Today estimate that about 80 % of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. India is a vast repository of medicinal plants that are used in traditional medical treatments[1]. Herbal drugs have great growth potential in global market. The World Health Organization has recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. There is a great demand for herbal medicines in the developed as well as developing countries because of their wide biological activities, higher safety margin than the synthetic drugs and lesser costs. There has been a major increase in their use in the last few years in the developed countries like Germany, France, European unions and U.S.A The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale[2]. The world market for herbal medicine, including herbal products and raw materials has been estimated to have an annual growth rate between 5 and 15%. Total global herbal drug market is estimated as US $62 billion and is expected to grow to US $5 trillion by the year 2050[3].

Herbal drugs have been used since ancient times as medicines for treatment of a range of diseases. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries. Over the past decade, interest in drugs derived from higher plants, especially the phytotherapeutic ones, has increased expressively. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants. Currently, the major pharmaceutical companies have demonstrated renewed interest in investigating higher plants as sources for new lead structures and also for the development of standardized

phytotherapeutic agents with proved efficacy, safety and quality. Herbal medicinal preparations are normally very popular in developing countries with a long tradition in the use of medicinal plants and also in some developed countries such as Germany, France, Italy and the United States where appropriate guidelines for registration of medicines exist. Phytomedicines are standardized herbal preparations consisting of complex mixtures of one or more plants which are used in most countries for the management of various diseases. Usually, the active principles responsible for their pharmacological action are unknown. One basic characteristic of herbal medicine is the fact that they normally do not posses an immediate or strong pharmacological action. For this reason, herbal medicines are not used for emergency treatment. Other characteristics of herbal medicines are their wide therapeutic use and great acceptance by the population. In contrast to modern medicines, herbal medicines are frequently used to treat chronic diseases. Herbal medicines are normally marketed as standardized preparations in the form of liquid, solid, or viscous preparations.

Compared with well-defined synthetic drugs, herbal medicines exhibit some marked differences, namely:

- ✓ The active principles are frequently unknown.
- ✓ Standardization, stability and quality control are feasible but not easy.
- ✓ The availability and quality of raw materials are frequently problematic.
- ✓ The well-controlled double-blind clinical and toxicological studies to prove their efficacy and safety are rare.
- ✓ The empirical use in folk medicine is a very important characteristic.
- ✓ They have a wide range of therapeutic use and are suitable for chronic treatments.
- ✓ The occurrence of undesirable side effects seems to be less frequent with herbal medicines, but well-controlled randomized clinical trials have revealed that they also exist.
- ✓ They usually cost less than synthetic drugs.

During the past decades, public interest in herbal medicines, has increased dramatically not only in developing countries but mainly in industrialized countries. This has increased international trade in herbal medicines enormously and has attracted most of the pharmaceutical companies, including the multinationals. The herbal medicine database indicates that the herbal medicine markets in Asia and Japan

reach $2.3 and 2.1 billion, respectively. The herbal medicine market has grown at an expressive rate worldwide[4]. Several important factors have contributed to the growth of this worldwide herbal market, among which the following may be mentioned:

- ✓ Preference of customers for natural therapies.
- ✓ Its concern regarding undesirable side effects of modern medicines and the belief that herbal drugs are free from side effects, since millions of people all over the world have been using herbal medicines for thousands of years.
- ✓ Its great interest in alternative medicines.
- ✓ The preference of populations for preventive medicine due to increasing population age.
- ✓ The belief that herbal medicines might be of effective benefit in the treatment certain diseases where conventional therapies and medicines have proven to be inadequate.
- ✓ Tendency towards self-medication
- ✓ Improvement in quality, proof of efficacy and safety of herbal medicines.
- ✓ High cost of synthetic medicines.

The general idea that herbal medicines are very safe and free from side effects is false. Plants have hundreds of constituents and some are very toxic such as the most cytotoxic and anti-cancer plant derived drugs, digitalis, the pyrolizidine alkaloids, ephedrine, phorbol esters, etc. However, the adverse effects of most herbal drugs are relatively less frequent when the drugs are used properly compared with synthetic drugs, but well-controlled clinical trials now confirm that they really exist. Two kinds of side effects have been reported for herbal medicines. The first, considered to be intrinsic to herbal drugs themselves, is mainly related to predictable toxicity, over-dosage and interaction with urea; drugs, as reported in herbal medicines. Thus many causes of allergic reactions have been reported for herbal medicines. On the other hands most of side effects reported for herbal drugs are extrinsic to the preparation and are selected to manufacturing problems such as misidentification of plants, lack of standardization, failure of good manufacturing practice, contamination, substitution and adulteration of plants, incorrect preparations and/or dosage, etc. Since 1978, more than 4000 herbal preparations have been submitted to pharmacovigilance in Germany

and most herbal drugs have been withdrawn from the market because of toxic effects and risks for human use.

Standardization of herbs and quality of herbal preparations

Herbal medicines usually contain a range of pharmacologically active compounds; in some cases it is not known which ingredients are important for the therapeutic effect[5]. Many herbalists believe that isolated ingredients have weaker clinical effects than whole plant extracts, a notion that would obviously require proof in each case. The multi-ingredient character of herbal medicines can render efficacy testing more complex than with synthetic drugs. One approach is to view the entire herbal extract as the active principle. To optimize the reproducibility of such studies, extracts need to be sufficiently characterized. This is often attempted through standardization according to a key constituent of the extract.

Plants contain several hundred constituents and some of them are present in very low concentrations. In spite of the modern chemical analytical procedures available, only rarely do phytochemical investigations succeed in isolating and characterizing the all secondary plant metabolites present in plant extract. Apart from this, plant constituents vary considerably depending on several factors that impair the quality control of herbal drugs. Quality control and standardization of herbal medicines involve several steps. However, the source and quality of raw materials play a pivotal role in guaranteeing the quality and stability of herbal preparations. Other factors such as the use of fresh plants, temperature, light, exposure, water availability, nutrients, period and time of collection, method of collecting, drying, packing, storage and transportation of raw materials, age and part of plant collected, etc., can greatly affect the quality and consequently the therapeutic value of herbal medicines. Some plant constituents are heat labile and the plants containing them need to be dried at low temperature. Also, other active principles are destroyed by enzymatic processes that continue for long period of time after plant collection. This explains why frequently the composition of herbal based drugs is quite variable. Thus, proper standardization and quality control of raw material and the herbal preparations themselves should be permanently carried out. In cases where active principles are unknown, marker substance should be established for analytical purposes. However, in most cases these markers have never been tested to see whether they really account for therapeutic action reported for the herbal drugs. As pointed out before, apart from these variable

factors others such as method of extraction and contamination with microorganisms, heavy metals, pesticides, etc., can also interfere with quality, safety and efficacy of drugs. For these reasons pharmaceutical companies prefer using cultivated plants instead of wild-harvested plants because they show similar variation in their constituents. Furthermore and certainly more relevant, when medicinal plants are produced by cultivation, the main secondary metabolites can be monitored and this permits the definition of the best period for harvesting.

The recent advances which occurred in the process of purification, isolation and structure elucidation of naturally occurring substances have made it possible to establish appropriate strategies for the analysis of quality and the process of standardization of herbal preparations in order to maintain as much as possible the homogeneity of the plant extract. Thin-layer chromatography, gas chromatography, high performance liquid chromatography, mass spectrometry, infrared-spectrometry, ultraviolet/visible spectrometry, etc. used alone or in combination, can be successfully used for standardization and to control the quality of both raw material and the finished herbal drugs. The growth of the herbal medicine market has attracted much interest on the part of the pharmaceutical companies, which has in turn stimulated the appearance of pre-clinical pharmacological studies and of well controlled and randomized clinical trials to prove their safety and efficacy[6].

During the twentieth century, the emphasis gradually shifted from extracting medicinal compounds, from plants to making these compounds or their analogues synthetically. Natural products were widely used as templates for structure optimization programs designed to make perfect new drugs, referred to by industry as new chemical entities. Despite the current preoccupation with synthetic chemistry as a vehicle to discover and manufacture drugs, the contribution of plants to disease treatment and prevention is still enormous. The greatest recent impact of plant-derived drugs was probably felt in antitumour area, where taxol, vinblastine, vincristine and camphothecin have dramatically improved the effectiveness of chemotherapy against some of the deadliest cancers[7].

Despite the complexity of herbal products, investigations of their efficacy are feasible and desirable, particularly vis-a-vis their popularity. Some herbal medicines are efficacious for certain indications. All herbal medicines are associated with safety issues which are often complex[8-10].

The most important pharmaceuticals derived from plants directly or precursors are listed in Table 1.

Table 1. Some of the economically important pharmaceuticals or their precursor derived from plants.

S.No	Name	Type	Source	Therapeutic uses
		Alkaloids		
1.	Atropine, hyoscyamine, Scopolamine	Tropane alkaloid	*Solanaceous species*	Anticholinergic
2.	Camptothecin	Indole alkaloid	*Camphotheca acuminate*	Antineoplastic
3.	Capsaicin	Phenylalkyl-amine alkaloid	*Capsicum species*	Topical analgesic
4.	Codeine, morpine	Opium alkaloid	*Papaver somniferum*	Analgesic, antitussive
5.	Cocaine	Cocaine alkaloid	*Erythroxylum coca*	Local anaesthetic
6.	Colchicine	Isoquinoline alkaloid	*Colchicum autumnale*	Antigout
7.	Emetine	Isoquinoline alkaloid	*Cephaelis ipecacuanha*	Antiamoebic
8.	Galanthamine	Isoquinoline alkaloid	*Leucojum aestivum*	Cholinesterase inhibitor
9.	Nicotine	Pyrrolidine alkaloid	*Nicotiana species*	Smoking cessation therapy
10.	Physostigmine	Indole alkaloid	*Physostigma venenosum*	Cholinergic
11.	Pilocarpine	Imidazole alkaloid	*Pilocarrpus jaborandi*	Cholinergic
12.	Quinine	Quinoline alkaloid	*Cinchona species*	Antimalarial
13.	Quinidine	Quinoline alkaloid	*Cinchona species*	Cardiac depressant
14.	Reserpine	Indole alkaloid	*Rauwolfia serpentina*	Antihypertensive, Psychotropic
15.	Tubocurarine	Bisbenzyl isoquinoline alkaloid	*Chondodendron tomentosum,*	Skeletal muscle relaxant

			Strychnos toxifera	
16.	Vinblastine, Vincristine Yohimbine	Bis-indole alkaloid, indole alkaloid	*Catharanthus roseus*	Antineoplastic, Aphrodisiac
Terpenes and steroids				
17.	Artemisnin	Sesquiterpine, Lactone	*Artemisia annua*	Antimalarial
18.	Diosgenin, hecogenin, Stigmasterol	Steroids	*Dioscorea species*	Oral contraceptives and hormonal drugs
19.	Taxol and other taxoids	Diterpines	*Taxus brevifolia*	Antineoplastic
Glycosides				
20.	Digoxin, digitoxin	Steroidal glycosides	*Digitalis species*	Cardiotonic
21.	Sennosides A and B	Hydroxy-anthracene glycosides	*Cassia angustifolia*	Laxative
Others and mixtures				
22.	Podophyllotoxin	Lignan	*Podophyllum peltatum*	Antineoplastic

Need of plant based drugs

The lack of reproducibility of activity for greater than 40% of plant extracts is one of the major obstacles in using plants in pharmaceutical discovery, despite the great diversity of compounds they synthesize. The activities detected in screens often do not repeat when plants are resampled and re-extracted. Moreover, the biochemical profiles of plants harvested at different times and locations vary greatly. In addition, the currently popular high-throughput drug discovery format favors single compounds over mixtures and is not compatible with complex plant extracts in which valuable bioactive molecules are obscured by pigments and polyhenols that interfere with screens. Equally important is the lack of efficient, rapid strategies to isolate and characterize a natural product from a plant extract. This is roughly equivalent to the lifetime of a high-throughput screen for a new target and is a prohibitively long time in an ever-accelerating lead discovery race. The new chemical entity paradigm of the twentieth century attempts to treat complex diseases with a single golden molecular bullet. The first flaw in this paradigm appeared relatively recently when problems of resistance to antimicrobial and anticancer drugs became apparent. The multifactorial nature of many complex diseases, such as diabetes, heart disease, cancer and psychiatric disorders is also an important consideration. Most of these diseases cannot be ascribed to a single genetic or environmental change but arises from a combination of genetic, environmental or behavioral factors. The future of herbal drugs depends on two factors: sustaining a favorable regulatory environment and developing technologies for the efficient discovery, development and manufacture of herbal drugs. At present majority of herbal drugs under development are derived from ethnobotanical sources and traditional medicinal uses[11].

Role of herbal drugs in diabetes

The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It is predicted that by 2030, India, China and the United States will have the largest number of people with diabetes[12]. Diabetes mellitus is categorized as a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The vast majority of cases of diabetes fall into two broad categories, Type 1 diabetes and Type 2 diabetes. The cause Type 1 diabetes is an absolute deficiency of insulin secretion. In type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin-secretory response[13]. Despite the great interest in the

development of new drugs to prevent the burden of complications associated with this disease and the raised interest in the scientific community to evaluate either raw or isolated natural products in experimental studies. Nevertheless, natural supplements are widely used around the world to treat diabetes, but medical research does not support their effectiveness. In these studies the low methodological quality, small sample size of tested patients, and limited number of trials deserve caution in the interpretation of the positive data and require further examination in high-quality trials. Nowadays, clinical treatment of diabetes targets both insulin deficiency and resistance and more recently the prevention of pancreatic cell function decline[14-18]. Medicinal plants are being looked up for the treatment of diabetes. Many conventional drugs have been derived from prototypic molecules in medicinal plants. To date, over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type II diabetes. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated.

Diabetes mellitus is a complex metabolic disorder resulting from either insulin insufficiency or insulin dysfunction. Type I diabetes (insulin dependent) is caused due to insulin insufficiency because of lack of functional beta cells. Patients suffering from this are therefore totally dependent on exogenous source of insulin while patients suffering from Type II diabetes (insulin independent) are unable to respond to insulin and can be treated with dietary changes, exercise and medication. Type II diabetes is the more common form of diabetes constituting 90% of the diabetic population[19]. A list of medicinal plants used in diabetes mellitus is given in Table 2.

Table 2. Medicinal plants used in Diabetes mellitus

S.No	Name of the plant	Parts used	Extracts used
1.	*Aegle marmelos*	Leaves Seeds Fruits	Ethanol extract Aqueous extract Aqueous extract
2.	*Aloe vera*	Leaves	Ethanol extract
3.	*Annona squamosa*	Leaves	Aqueous extract
4.	*Averroha bilimbi*	Leaves	Aqueous extract, Butanol extract, Ethyl acetate extract
5.	*Baccharis trimera*	Leaves	Aqueous extract, Butanol extract, Ethanol extract
6.	*Bryophyllum pinnatum*	Leaves	Aqueous extract
7.	*Cichorium intybus*	Whole plant	Ethanol extract
8.	*Chamaemelum nobile*	Leaves	Aqueous extract
9	*Egyptian Morus alba*	Leaves	Alcoholic extract
10.	*Eugenia jambolana*	Fruit-pulp, seeds	Aqueous, Ethanol extract
11.	*Allium sativum*	Bulbs	Ethanol extract
12.	*Mangifera indica*	Stem-bark	Aqueous extract
13.	*Momordica charantia*	Leaves, Seeds	Aqueous extract
14.	*Piper bettle*	Leaves	Aqueous, Ethanol extract
15.	*Psidium guajava*	Leaves	Aqueous extract
16.	*Raphanus sativus*	Whole plant	Aqueous extract
17.	*Retama raetum*	Whole plant	Aqueous extract
18.	*Salavia officinalis*	Leaves	Aqueous extract
19.	*Scoparia dulcis*	Whole plant	Aqueous extract
20.	*Strobilanthes crispus*	Leaves	Aqueous extract
21.	*Syzygium cordatum*	Leaves	Aqueous extract
22.	*Syzygium cumini*	Leaves, Seeds	Aqueous extract, Ethanol extract, Butanol extract
23.	*Taxus yunnanensis*	Woods	Aqueous, Ethanol extract

24.	*Terminalia chebula*	Seeds	Chloroform extract
25.	*Terminalia superba*	Stem bark	Methanol extract, Methylene chloride extract
26.	*Trema orientalis*	Stem bark	Aqueous extract
27.	*Tremella mesenterica*	Fruits	Polysaccharides
28.	*Triticum repens*	Rhizomes	Aqueous extract
29.	*Viscum album*	Whole plant	Aqueous, Ethanol extract
30.	*Zizyphus spina-christi*	Leaves	Butanol extract

Metabolic Dearrangements in diabetes

Type I and Type II diabetes frequently co-occur in the same families, suggesting common genetic susceptibility. Such mixed family history is associated with an intermediate phenotype of diabetes: insulin resistance and cardiovascular complications as well as lower C-peptide concentrations in type II diabetic patients[20]. The autoimmune nature of type I diabetes has been intensively investigated, and it has long been assumed that the pathogenesis of the disease can be explained by an interplay between genetics and environment. The pathogenesis of type I diabetes is that, genetically predisposed individual and environmental factors trigger an autoimmune process (activation of T lymphocytes reactive to islet cell antigens) that leads to the destruction of islet cells and insulin deficiency**.**

Type II diabetes, previously known as NIDDM or adult-onset diabetes, is the most prevalent form of diabetes, accounting for over 90% of all cases of diabetes. Type II diabetes is characterized by varying degree of insulin resistance and insulin deficiency. It is thought that earliest defect in the pathogenesis of type II is impaired insulin action or insulin resistance. Resistance to the action of insulin will result in impaired insulin mediated glucose uptake in the periphery (by muscle and fat), incomplete suppression of hepatic glucose output and impaired triglyceride uptake by fat. To overcome the insulin resistance (and therefore prevent abnormal fuel metabolism and maintain normal glucose and lipid levels), beta islet cells will increase the amount of insulin secreted. Therefore two defects are necessary for development of type II diabetes insulin resistance and insulin deficiency[21].

Type I diabetes (Insulin dependent diabetes mellitus-IDDM)

Type I diabetes is the most severe type of diabetes, requiring daily insulin injections on a life long basis. The etiology of Type I diabetes is not well understood. This type of diabetes results from selective destruction of the insulin producing β cells of the pancreatic islets of Langerhans, a process that is immunologically mediated and occurs in genetically susceptible individuals. The islet β-cells are destroyed by an autoimmune response mediated by T lymphocytes (T cells) that react specifically to one or more β-cell proteins[22].

Type II diabetes (Non insulin dependent diabetes mellitus -NIDDM)

Type II is the most common form of diabetes and is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature[11]. Type II diabetes mellitus is a group of disorders characterized by hyperglycaemia and associated with microvascular (i.e., retinal, renal, possibly neuropathic), macrovascular (i.e., coronary, peripheral vascular) and neuropathic (i.e., autonomic, peripheral) complications. Hyperglycaemia is produced by lack of endogenous insulin, which is either absolute, as in type I diabetes mellitus, or relative, as in type II diabetes mellitus. Relative insulin deficiency usually occurs because of resistance to the actions of insulin in muscle, fat, and the liver and an abnormality results in decreased glucose transport in muscle, elevated hepatic glucose production, and increased breakdown of fat[23]. The natural history of Type II diabetes has four stages. The first stage begins at birth, when glucose homeostasis is normal but individuals are at risk for Type II diabetes because of genetic polymorphisms (diabetogenic genes). During stage II, decrease in insulin sensitivity emerges probably as a result of a genetic predisposition and lifestyle (environmental), which are initially compensated by an increase in β-cell function, so that glucose tolerance remains normal. Later on, both the β-cell function and insulin sensitivity deteriorate, so that when challenged, as during a glucose tolerance test or a standardized meal, postprandial glucose tolerance becomes abnormal (stage of impaired glucose tolerance). At this point, β-cell function is clearly abnormal, but sufficient to maintain normal fasting plasma glucose concentrations. In stage III, as a result of further deterioration in β-cell function and increased insulin resistance, fasting plasma glucose can increase due to an increase in basal endogenous glucose production, but the patient is still asymptomatic. Finally in stage IV, as a result of further deterioration in β-cell function, both fasting and postprandial blood glucose levels reach clearly

diabetic levels and the patient becomes symptomatic[24]. Pathophysiology of hyperglycaemia is given in Figure 1:

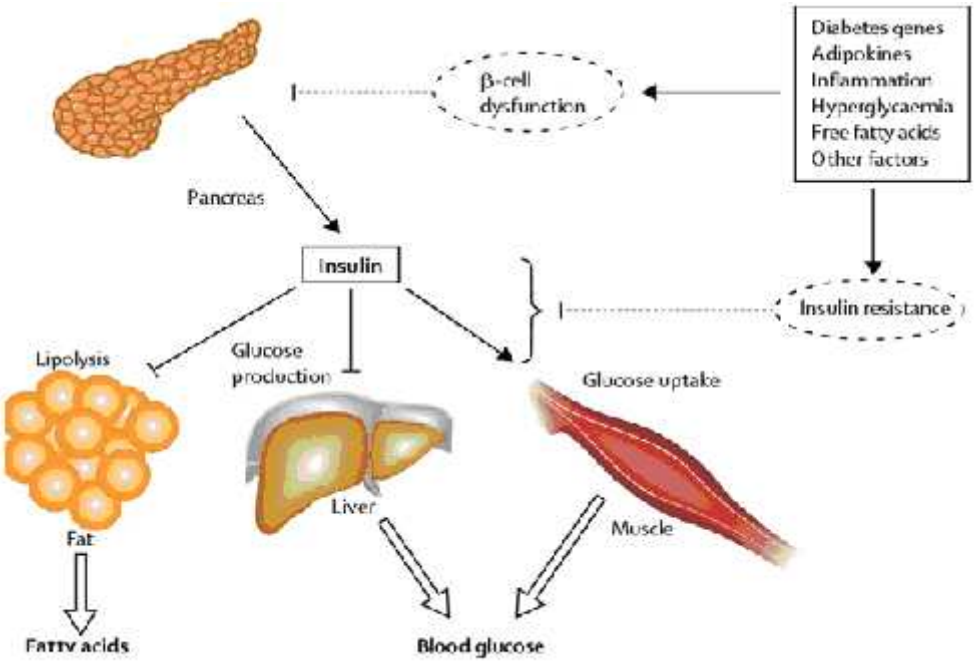


Figure 1. Pathophysiology of hyperglycaemia

Gestational diabetes

Gestational diabetes (or gestational diabetes mellitus, GDM) is a condition in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy. Gestational diabetes is formally defined as "any degree of glucose intolerance with onset or first recognition during pregnancy". No specific cause has been identified, but it is believed that the hormones produced during pregnancy reduce a woman's sensitivity to insulin, resulting in high blood glucose levels. Gestational diabetes generally has few symptoms and it is most commonly diagnosed by screening during pregnancy. Diagnostic tests detect high levels of glucose in blood samples. Babies born to mothers with gestational diabetes are at increased risk of complications, primarily growth abnormalities and chemical imbalances such as low blood glucose. Gestational diabetes is a reversible condition and women who have adequate control of glucose levels can effectively decrease the associated risks and give birth to healthy babies. Women with gestational diabetes are at increased risk of developing type II diabetes mellitus after pregnancy, while their offspring are prone to developing childhood obesity, with type II diabetes later in life.

Most patients are treated only with diet modification and moderate exercise but sometimes anti-diabetic drugs, including insulin becomes necessary[25].

Management of diabetes

The goals of treatment for diabetes are to reduce and control blood glucose levels, relieve the symptoms of the disease, and prevent complications. Long-term control of blood glucose levels can be assessed by measuring the extent to which glucose is bound to the A_{1c} component of hemoglobin. This glycosylated hemoglobin value is expressed as a percentage and should be less than 7% in patients with diabetes. Numerous studies have demonstrated that intensive treatment and careful control of blood glucose levels can reduce the risk of complications from diabetes. The successful management of diabetes requires lifestyle changes for the patient (e.g., diet, exercise, self-monitoring of blood and possibly urine). All patients with type I diabetes require insulin injections. Patients with type II diabetes who have multiple symptoms are severely hyperglycaemic, or have ketosis (increased ketone production due to the breakdown of fat for energy) also should use insulin injections. Other patients with type II diabetes may attempt to control their diabetes through diet and exercise for a 3-month trial period. However, only 10% of patients with type II diabetes can control their blood glucose levels with diet and exercise alone. Various oral antidiabetic agents (or insulin) may be used in conjunction with diet and exercise to control blood glucose levels in these patients.

Insulin

Injections of insulin help cells take in glucose, thereby reducing blood glucose levels. Insulin is usually given as intermediate-acting insulin or a mixture of an intermediate-acting insulin plus a short-acting or rapid-acting insulin. Injections should be administered at least twice daily. Some patients may require three or more daily insulin injections or continuous subcutaneous infusions with an insulin pump[26].

Oral Antidiabetic Agents

Classes of oral antidiabetic agents include sulfonylureas, biguanides, alpha-glucosidase inhibitors, meglitinides, and thiazolidinediones. Each class has a unique mechanism of action, pharmacological effects and adverse-effect profile. If monotherapy with an oral antidiabetic agent (plus diet and exercise) is ineffective in controlling blood glucose levels, adding a second antidiabetic agent (preferably with a different mechanism of action) or insulin is recommended. Using two or more oral

antidiabetic agents with different mechanisms of action may provide an additive blood glucose-lowering effect and minimize adverse effects. If combination therapy is not effective, insulin monotherapy is recommended[27].

Sulfonylureas

Sulfonylureas are the best-established oral antidiabetic agents and are a rational choice for initial drug therapy[28]. They act primarily to increase insulin secretion by pancreatic beta cells and can cause hypoglycaemia. Weight gain also can occur with Sulfonylurea therapy.

Biguanides

Currently, metformin is the only drug in the biguanide class. Metformin reduces blood glucose concentrations by increasing glucose uptake in the peripheral muscles and decreasing the amount of glucose produced and released in the liver. The most common adverse effects from metformin are diarrhea and other gastrointestinal disorders. Metformin is contraindicated in patients with renal impairment because of the risk of lactic acidosis, a potentially serious complication.

Alpha-Glucosidase Inhibitors

The alpha-glucosidase inhibitors, acarbose and miglitol inhibit the action of intestinal enzymes that break down carbohydrates. These oral antidiabetic agents delay glucose absorption and are particularly useful for patients with postprandial hyperglycaemia (high blood glucose levels after eating). However, they are not as effective as sulfonylureas and biguanides in providing long-term control of blood glucose levels[29]. Alpha glucosidase inhibitors do not cause hypoglycemia or weight gain. However, they can cause flatulence, diarrhea, and abdominal cramps[30]. Flow chart for management of diabetes is given in Figure 2.

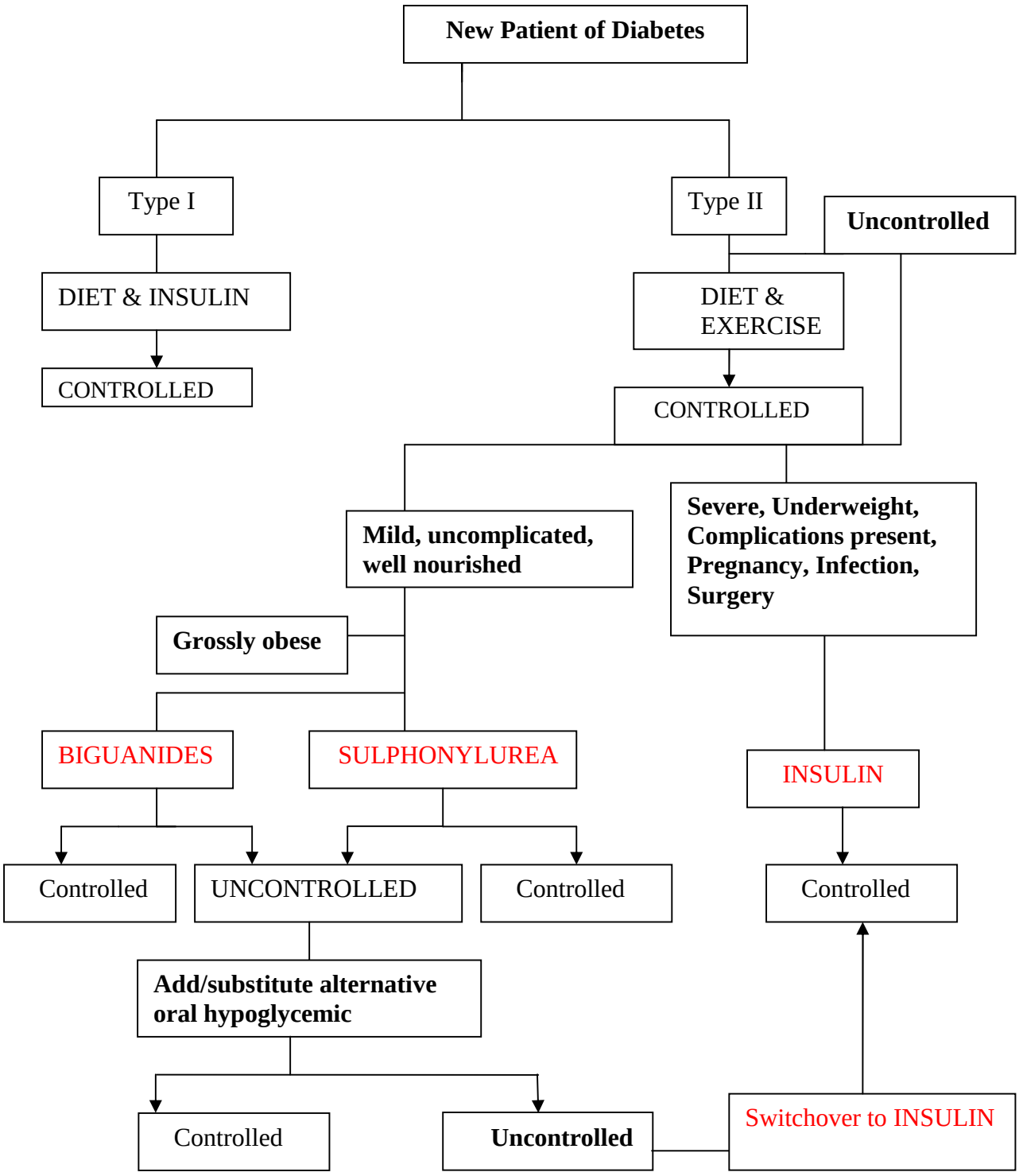


Figure 2. Flow chart of management of diabetes mellitus

2. **Review of Literature**

INSULIN

Insulin was the first protein for which an amino acid sequence was determined. It consists of two peptide chains, A and B, of 21 and 30 amino acid residues, respectively. Insulin is the main hormone controlling intermediary metabolism. Most cells of the body have insulin receptors which bind with insulin in the circulation. Structure of Insulin monomer is given in Figure 3. Structure of Insulin hexamer is given in Figure 4. Chained structure of insulin is given in Figure 5.

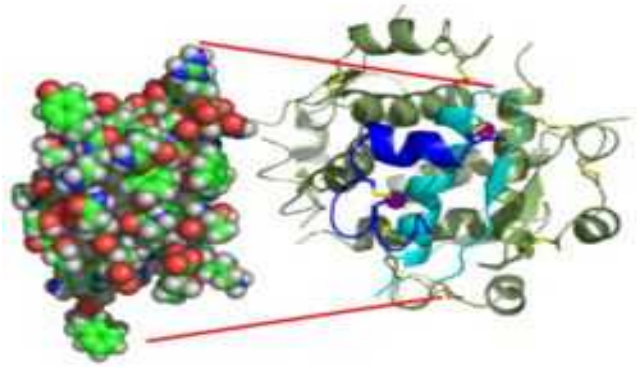


Fig. 3. Structure of Insulin monomer

Insulin is synthesized as a precursor in the rough endoplasmic reticulum[31]. Preproinsulin is transported to Golgi apparatus where it undergoes proteolytic cleavage first to proinsulin and then to insulin plus a fragment of uncertain function called C-peptide. Insulin and C-peptide are stored in granules in β-cells and are normally cosecreted by exocytosis in equimolar amounts together with smaller and variable amounts of proinsulin[32,33]. The main factor controlling the synthesis and secretion of insulin is the blood glucose concentration. Insulin is the main hormone controlling intermediary metabolism, having actions on liver, muscle and fat. Its overall effect is to conserve energy by facilitating the uptake and storage of glucose, amino acids and fats after a meal. It reduces blood glucose and fall in plasma insulin level stimulates blood glucose release. Effect of insulin on glucose uptake and metabolism is given in Figure 6. Insulin binds to its receptor, which in turn starts many protein activation cascades. These cascades include: translocation of Glut-4 transporter to the plasma membrane and influx of glucose, glycogen synthesis, glycolysis and fatty acid synthesis.

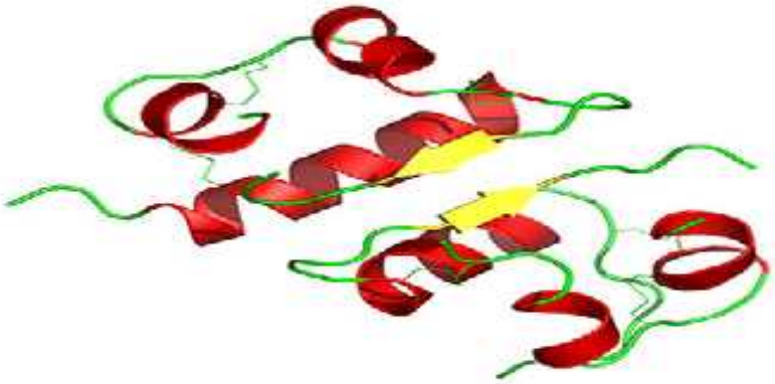


Fig. 4. Structure of Insulin hexamer

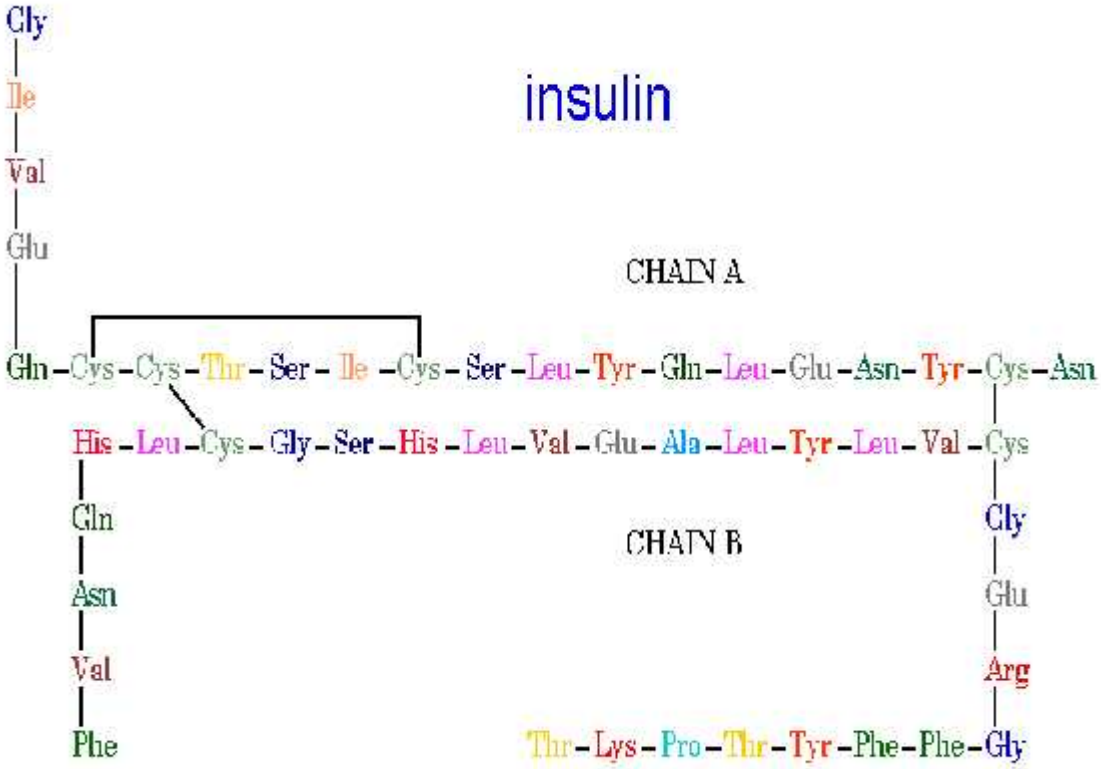


Fig. 5. Chained Structure of insulin

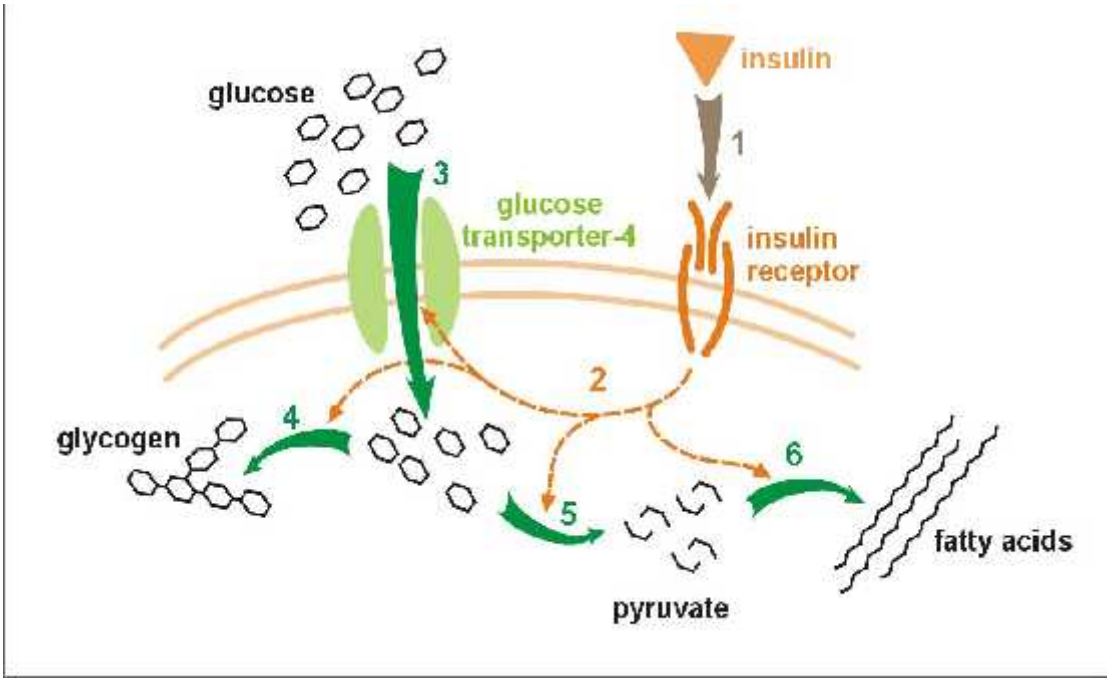


Fig. 6. Effect of insulin on glucose uptake and metabolism.

Effect of insulin on carbohydrate metabolism

Insulin influences glucose metabolism in liver by inhibiting glycogenolysis (glycogen breakdown) and gluconeogenesis (synthesis of glucose from non-carbohydrate sources) while stimulating glycogen synthesis[34]. It also increases glucose utilization, but overall effect is to increase hepatic glycogen stores. The main effect of insulin in muscle, is to increase facilitated transport of glucose via a glucose transporter called Glut-4, and stimulate glycogen synthesis and glycolysis. Insulin increases glucose uptake by Glut-4 in adipose tissue as well as in muscle, by enhancing glucose metabolism. One of the main end products of glucose metabolism in adipose tissue is glycerol, which is esterified with fatty acids to form triglycerides.

Effect of insulin on fat metabolism

Insulin increases fatty acid as well as triglyceride synthesis in adipose tissue and liver. It inhibits lipolysis, partly via dephosphorylation of lipases. It also inhibits the lipolytic actions of adrenaline, growth hormone and glucagon by opposing their action on adenylate cyclase**.**

Effect of insulin on protein metabolism

Insulin stimulates uptake of amino acids into muscle and increases protein synthesis. It also decreases protein catabolism and inhibits oxidation of amino acids in the liver.

Mechanism of action of insulin

Insulin binds to a specific protein receptor on the surface of its target cells. The receptor is a large transmembrane glycoprotein complex consisting of two α and two β-subunits. The α-subunits are entirely extracellular and each carries an insulin-binding state, where as the β-subunits are transmembrane proteins with tyrosine kinase activity. This activity is suppressed by the α-subunits, but insulin binding causes a conformational change that activates the tyrosine kinase activity of the β subunits which act on each other and on other target proteins. 10 % of receptors are occupied at maximum concentration of insulin. Occupied receptors aggregate into clusters, which are subsequently internalized in vesicles, resulting in down regulation. Internalized insulin is degraded in lyosomes, but the receptors are recycled to the plasma membrane[35]. Mechanism of insulin release is given in Figure 7.

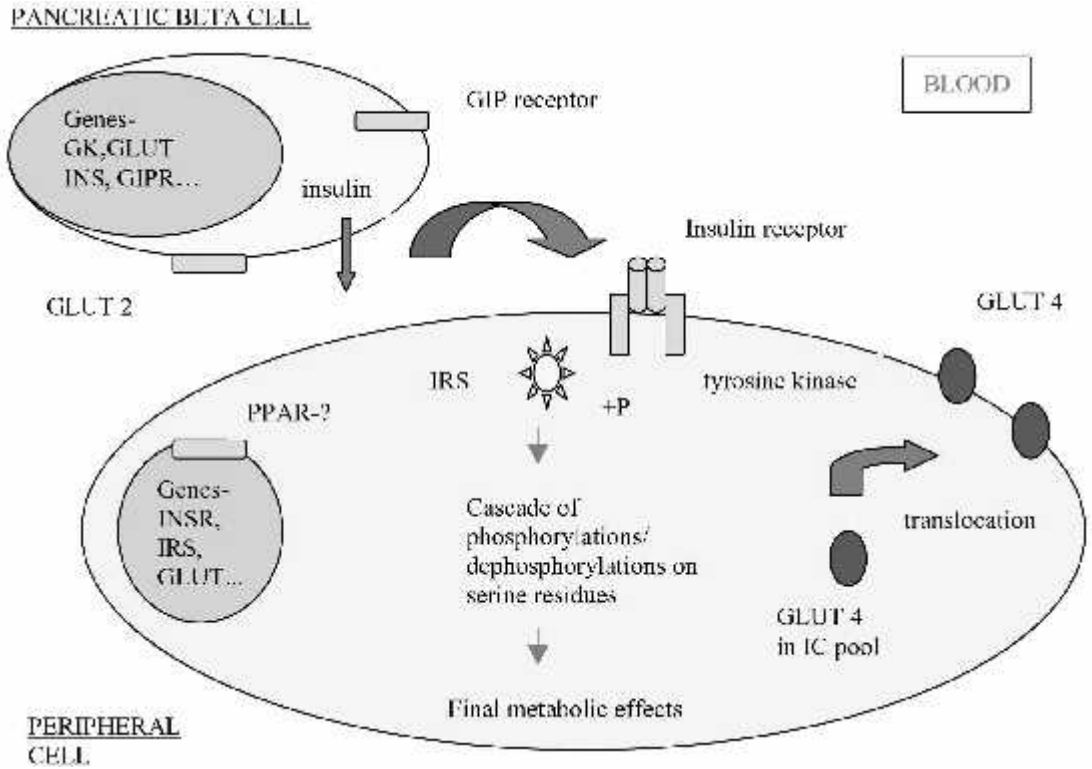


Fig. 7. Mechanism of insulin release

ATP-sensitive potassium channels ('K_{ATP}') determine the resting membrane potential in β-cells. Glucose enters β-cells via a membrane transporter called Glut-2, and its subsequent metabolism via glucokinase and glycolysis increases intracellular ATP. This blocks K_{ATP} , causing membrane depolarization and opening of voltage dependent calcium channels, leading to Ca^{2+} influx. The Ca^{2+} signal induces insulin secretion, but only in the presence of amplifying messengers including diacylglycerol (DAG), non-esterified arachidonic acid (which facilitates further Ca^{2+} entry) and 12-lipoxygenase products of arachidonic acid mainly 12S-hydroxyeicosatetraenoic acid (12-S-HETE). Phospholipases are commonly activated by Ca^{2+}, but free acrachidonic acid is liberated in β-cells by an ATP-sensitive Ca^{2+} insensitive ('ASCI') phospholipase $A_{2.}$

Consequently, in β-cells Ca^{2+} entry and arachidonic acid production are both driven by ATP, linking cellular energy status to insulin secretion. Many gastrointestinal hormones stimulate insulin secretion, including gastrin, secretin, cholecystokinin, gastric inhibitory polypeptide (GIP), glucagon-like peptide (GLP) and GLP_1 (the amide of a fragment of GLP). Insulin release is inhibited by the sympathetic nervous system. Adrenaline increases blood glucose by inhibiting insulin release and by promoting glycogenolysis via β_2–adrenocepters in striated muscle and liver. Several peptides, including somatostatin, galanin (an endogeneous ATP-sensitive potassium channel activator) and amylin, also inhibit insulin release. Mechanism of insulin dependent glucose release is given in Figure 8.

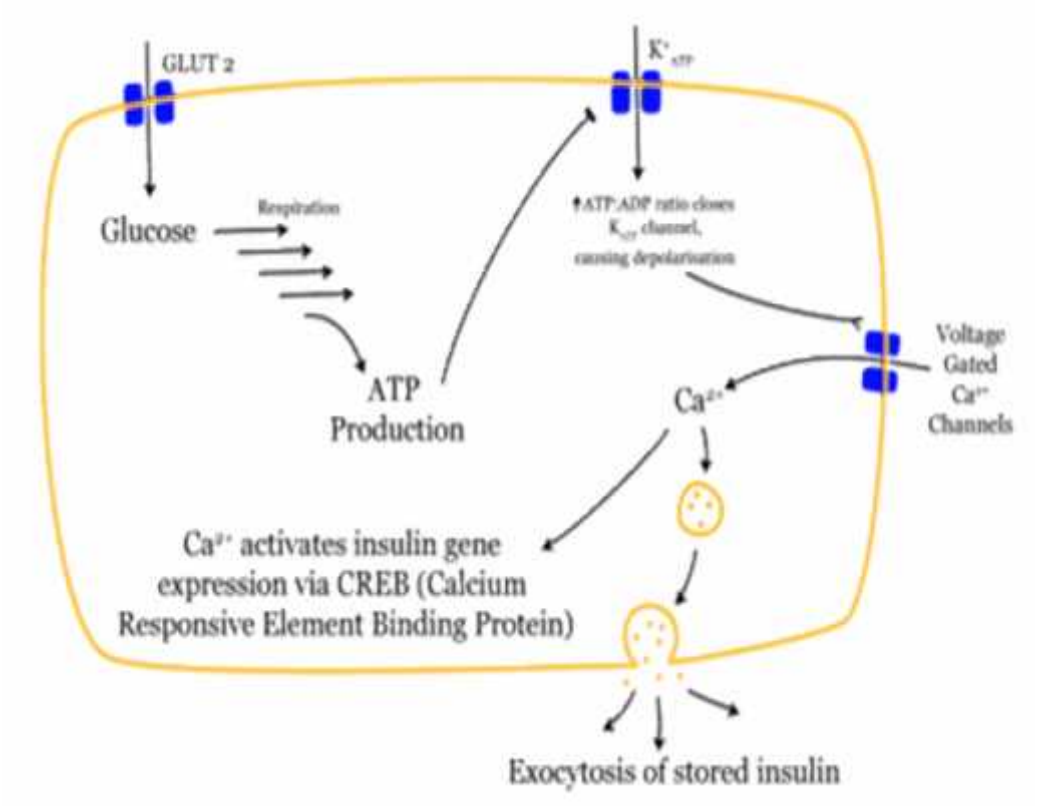


Fig. 8. Mechanism of glucose dependent insulin release

Oral medication used in treatment of Diabetes

The following Oral medication used in treatment of diabetes[36-41].

Repaglinide

It is a meglitinide that stimulates insulin release by pancreatic beta cells, although it is not a sulfonylurea. Repaglinide is indicated as monotherapy, but if blood glucose control cannot be achieved with repaglinide, then given in combination with metformin. Repaglinide and metformin together act synergistically to reduce glucose levels to lower levels than can be achieved with either drug alone. Repaglinide usually is taken two to four times a day, within 30 minutes before meals. Adverse effects include hypoglycemia and weight gain. Patients who are elderly, or malnourished or who have adrenal, pituitary, or hepatic insufficiency are particularly susceptible to hypoglycaemic reactions. Causes of Hyperglycaemia and action of oral antidiabetic agents is given in Figure 9.

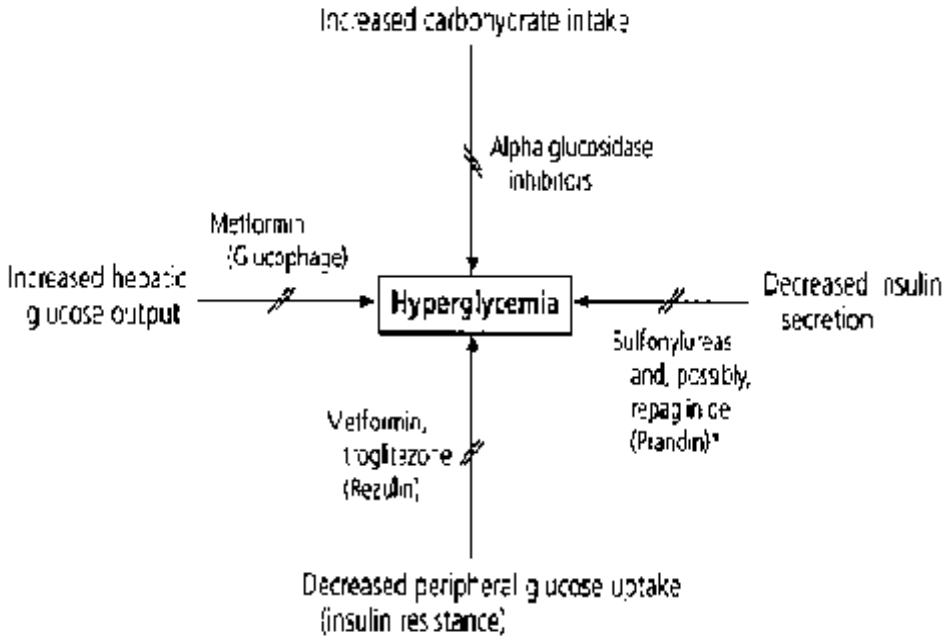


Fig. 9. Causes of Hyperglycaemia and action of oral antidiabetic agents

Nateglinide

It is a non-sulfonylurea with a mechanism of action similar to that of repaglinide; it stimulates the release of insulin by pancreatic beta cells. Like repaglinide, nateglinide is indicated for use as monotherapy or in combination with metformin. Nateglinide and metformin have a synergistic blood glucose lowering effect. Nateglinide is taken three times daily, within 30 minutes before meals. Hypoglycaemia and weight gain can occur during nateglinide therapy.

Thiazolidinediones

Thiazolidinediones are the newest class of antidiabetic agents. They increase insulin sensitivity and action in liver, muscle, and fatty tissues. Thiazolidinediones reduce blood glucose concentrations and improve the lipid profile. These agents do not stimulate insulin secretion or cause hypoglycaemia. However, they can cause weight gain. The risk of severe liver toxicity appears to be lower with the newer thiazolidinediones, rosiglitazone and pioglitazone. Thiazolidines bind to gamma form of peroxisome proliferator-activated receptors (PPARγ). This stimulates peripheral adipocytes to increase their uptake of fatty acids, which leads to reduction in the fat stored in the muscle, liver and visceral fat deposits. The thiazolidinediones also lead to an increase in secretion of adiponectin and decrease in production of resistin and tumor necrosis factor.

Glibenclamide

Glibenclamide is an antidiabetic drug belongs to category of Sulphonylureas included in WHO Model list of Essential Medicines. The drug works by inhibiting ATP-Sensitive Potassium channels in Pancreatic Beta Cells. This inhibition causes cell membrane depolarization, opening of Voltage dependent Calcium Channels thus triggering an increase in intracellular calcium ions into the beta cell which stimulates Insulin release.

Chemicals used for inducing diabetes mellitus

The following chemicals are generally used for inducing diabetes[42]

1 Alloxan

2 Steptozotocin

3. Vacor (N-3- Pyridylmethyl-N –P-nitrophenylurea)

4. Dithizone

5. 8-hydroxyquinolone

6. Cyproheptadine

7. Hexamethyl-melamine

Alloxan (2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative. It is present as alloxan hydrate in aqueous solution. Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus[43-45].

Streptozotocin (STZ) is an antibiotic derived from *Streptomyces achromogenes* and structurally is a glucosamine derivative of nitrosourea. Its nitrosourea moiety is responsible for beta cell toxicity, while deoxyglucose moiety facilitates transport across cell membrane. It induces chemical diabetes in a wide variety of animal species through a massive destruction of Beta cells of the islets of langerhans and resulting in a reduced synthesis and release of Insulin[46,47]. Structure of Streptozotocin is given in Figure 10.

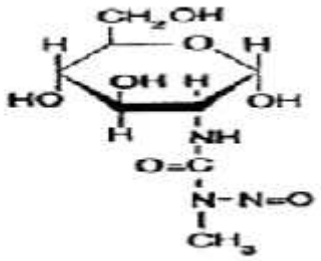


Fig. 10. Structure of streptozotocin

Diabetogenic activity of Streptozotocin

Streptozotocin causes alkylation or breakage of DNA strands and a consequent increase in activity of Poly-ADP-ribose synthetase, an enzyme depleting Nicotinamide adenine dinucleotide in Beta cells finally leading to energy deprivation and death of beta cells is reported. Streptozotocin enters the pancreatic Beta-cell via a glucose transporter-GLUT2. Streptozotocin is a preferred agent to induce experimental diabetes since it has some advantages over Alloxan such as, relatively longer half life (15 min), sustained hyperglycaemia for longer duration and the development of well characterized diabetic complications with fewer incidences of ketosis as well as mortality. Furthermore, STZ induces activation of poly adenosine diphosphate ribosylation, an enzyme depleting Nicotinamide in beta cells and nitric oxide release. As a result of STZ action, pancreatic Beta-cells are destroyed by necrosis.

Plant profile

Coldenia procumbens

Botanical name: *Coldenia procumbens* Linn.
Synonym: Tripakshi, Tripunkhi

Coldenia procumbens is a weed growing throughout India in moist places. It is distributed in sandy places, beaches and dry cultivated ground. It is a hygroscopic species which prefer black soil, mild moisture in and around lake. It grows mainly in Tamil Nadu, Andhra Pradesh and Gujarat in India[48]. A photograph of *Coldenia procumbens* is given in Figure 11.

Vernacular names

Hindi: Tripungkee, tripungki, tripunkhi, tripungki

Sanskrit: Tripakshee, tripakshi, tripakshi, tripakshi

Tamil: Serupadi, sirupadi, cheruppadi

Telugu: Hamapadi, hamsapdu, chepputattaku, hamsa padu, bukkinaku

Fig. 11. ***Coldenia procumbens***

Description

Leaves are alternate and petiolate. Branches are procumbent spreading flat on ground. Flowers were small, white, solitary hidden among leaves. A procumbent annual herb, stems are up to 40 cm. long, radiating, angular or laterally compressed, much branched, densely hairy to villous. Leaves are with petiole 1-5 mm. long, on the first ones up to 40 mm. long and lamina 4-23 x 3-15 mm. (on the first leaves 25-43 x 15-32 mm.) Leaves are usually oblong, sometimes circular, ovate, elliptic or obovate, with appresed hairs diverging from the nerves and converging to the teeth apex above, densly glandular- hairy below, apex rounded base assymetrical, cuneate on one side, obtuse to truncate on other, margins deeply crenate to lobed. Flowers are subsessile. Calyx is 2.5-3.0 mm. long, a little accrescent in fruit, villous outside and inside, lobes are ovate-lanceolate, subequal. Corolla is 1.5-2.2 mm long, narrowly conical, calyptriform, early deciduous, white, glabrous. Stamens inserted from the base of the corolla tube, anthers are suborbicular to broadly elliptic, filaments as long as the anthers, glabrous. Ovary is ovate-pyramidal, glandular-pubescent, styles are slightly united at base, early concealed by the apical ptoturbeances of the fruit. Fruit are 4-5 mm wide, depressed-ovoid, 4-lobed, beaked and with irregular proturbeances, glandular-hairy, brownish dividing at first into two pairs of nutlets, later into 4 nutlets with the ventral surface angular.[48-50]

Chemical constituents

Coldenia procumbens contains carbohydrates, tannins, proteins, steroids and flavonoids[50-52]. Powder microscopy and Microchemical tests also revealed the presence of the some chemical nature in the leaves.[53]

Ethnobotanical uses

It is used in treatment of fever, headache and rheumatic swellings. Root is used in the form of decoction for impaired digestion and leucorrhoea.

Biological activities

The plant possesses anti-inflammatory activity, analgesic activity and CNS depressant activity. The benzene, chloroform, acetone, and ethanol extracts of the leaves of *Coldenia procumbens* in the dose of 200mg/kg exhibited analgesic activity.[54,55]

The ethanol extract of aerial parts of *Coldenia procumbens* in the dose of 150 mg/kg, produced significant anti-inflammatory activity in male wistar rats.[56] The

ethanol extract of whole *Coldenia procumbens* plants showed depression of the central nervous system in mice and prolongation of the pentobarbital-induced sleeping time in rats[57].

3. AIM AND PLAN OF WORK

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia, glycosuria, hyperlipemia, negative nitrogen balance and ketonemia. A wide-spread pathological change is thickening of capillary basement membrane, increase in vessel wall matrix and cellular proliferation resulting in vascular complications like lumen narrowing, early atherosclerosis, sclerosis of glomerular capillaries, retinopathy, neuropathy and peripheral vascular insufficiency. There is significant increase in blood glucose levels, blood cholesterol levels and blood triglycerides levels. Synthetic drugs available for the treatment of diabetes results in large number produce adverse effects after prolonged duration.

There are many herbal remedies suggested for diabetes and diabetic complications. Medicinal plants form the main ingredients of the herbal remedies. Several plants have been screened for blood glucose lowering activities on experimental animals. Individual plants as well as herbal formulations were reported as hypoglycaemic and antidiabetic agents. However, very few plants have been studied in depth for the blood glucose lowering effect and mechanism of action for development as an antidiabetic agent. The plants such as *Allium cepa, Allium sativum, Syzygium cumini, Momordica charantia, Gymnema sylvestre, Pterocarpus marsupium, Coccinia indica, Trigonella foenum graceum, Psidium guajava and Aegle marmelos* have attracted more attention of scientists in recent years. *Coldenia procumbens* has been used for treatment of diabetes in folklore medicine. About 10-15 leaves are boiled in cup of water and used for diabetes mellitus.

Very little work has been reported on phytochemical Pharmacological screening of *Coldenia procumbens*. Hence, it is therefore decided to investigate the antidiabetic potential of leaves of *Coldenia procumbens*.

Qualitative chemical tests are proposed to be carried out in the 90% ethanol extract, 50% ethanol extract and aqueous extract in order to find out the chemical constituents.

Antidiabetic studies are proposed to be carried out using rats in three different models. The models proposed are:

1) Fasting glucose model
2) Oral glucose tolerance test (OGTT) in rats and

3) Streptozotocin induced diabetes model in rats.

Blood glucose levels, total cholesterol levels, and triglyceride levels would be estimated to asses the antidiabetic activity of the selected plant extracts.

Plan of work

The plan of present work will be as follows:

Phase: 1

Collection and authentication of plant material.

Phase: 2

a. Preliminary phytochemical studies.
b. Preparation of extracts
    i. Preparation of 50% ethanol extract
    ii. Preparation of 90% ethanol extract
    iii. Preparation of aqueous extract
c. Qualitative chemical tests

Phase: 3

Antidiabetic evaluation of *Coldenia procumbens*

a. Fasting glucose model
b. Oral glucose tolerance test (OGTT) in rats
c. Streptozotocin induced diabetic model in rats

Phase: 4

Assessment

a. Estimation of Blood glucose levels
b. Estimation of Total cholesterol levels
c. Estimation of triglyceride levels

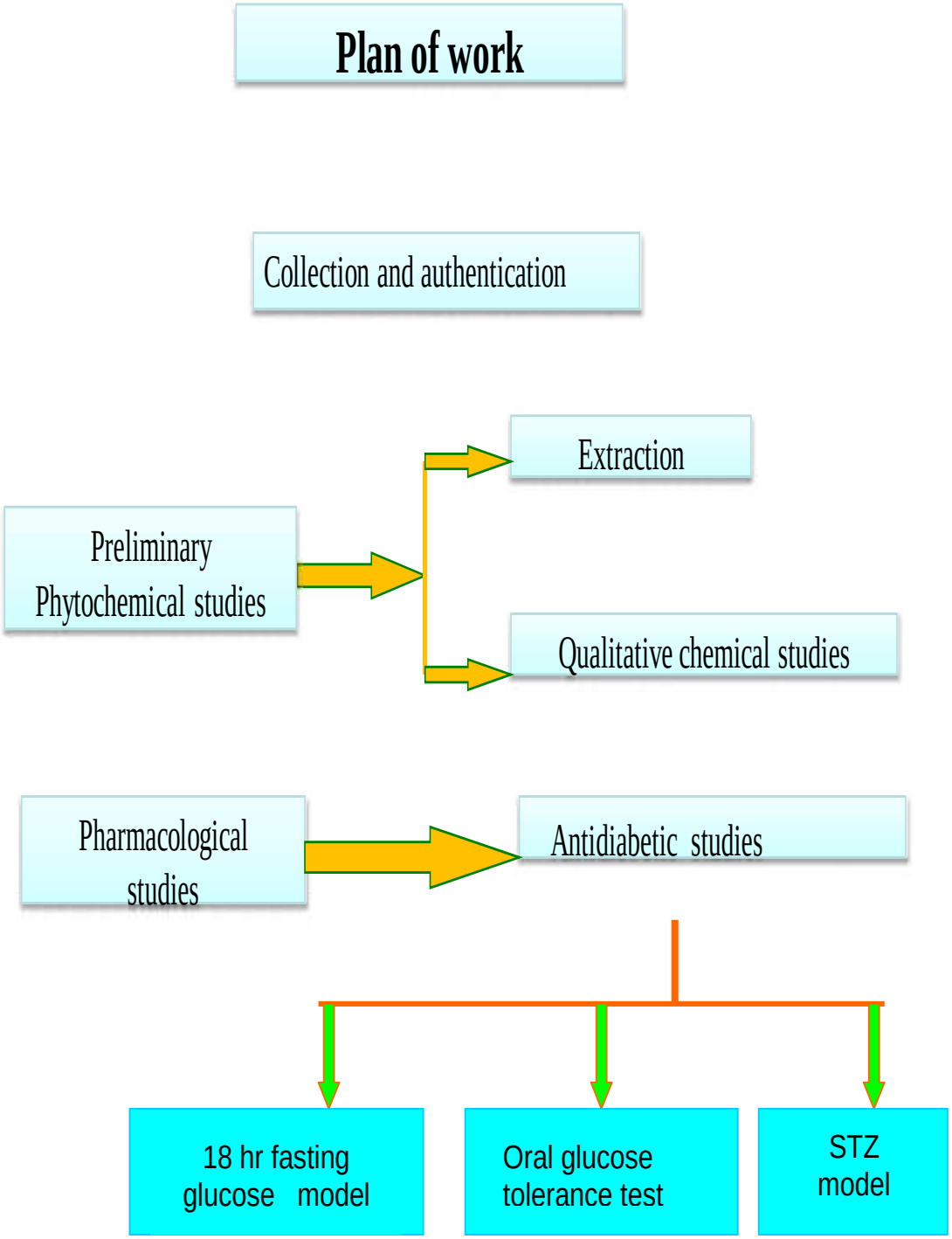


Fig. 12. Schematic representation of plan of work

4. Experimental Work

Chemicals and Solvents

All diagnostic kits were purchased from Bayer Diagnostics India Ltd., Baroda, Gujarat. Streptozotocin was purchased from Sigma Aldrich Co. India. Assay procedures were followed as per instructions of the manufacturers. All other chemicals used in present study were of analytical grade. Blood Glucose levels, Total Cholesterol levels and Triglycerides levels were measured by using Auto Pak.

Collection and Authentication of Plant materials

The plant, *Coldenia procumbens* was collected from Virrupalli village of Coimbatore district, Tamilnadu and authenticated by Prof. P.Jayaraman, Plant Anatomy Research Centre Pharmacognosy Institute, Chennai, India. Leaves of the plants were collected; shade dried, powdered and packed in an air tight container.

Extraction

Preparation of extracts

Preparation of 50% ethanol extract

200 gm of the air dried and finely ground plant material was extracted with 50% ethanol by cold maceration in a round bottom flask for 24 hrs. After 24 hrs, it was filtered through Whatman filter paper and the extract was collected in a round bottom flask. The process was repeated using marc for another two times and all the three filtrates were collected and combined. The solvent was removed by distillation and concentrated *in vacuo* in a rotary evaporator. The dried extract was placed in a desiccator and used for further studies. The colour, consistency and the percentage yield of the extract was recorded in Table 6.

Preparation of 90% ethanol extract

200 gm of the air dried and finely ground plant material was extracted with 90% ethanol by cold maceration in a round bottom flask for 24 hrs. After 24 hrs, it was filtered through Whatman filter paper and the extract was collected in a round bottom flask. The process was repeated using marc for another two times and all the three filtrates were collected and combined. The solvent was removed by distillation and concentrated *in vacuo* in a rotary evaporator. The dried extract was placed in a desiccator and used for further studies. The colour, consistency and the percentage yield of the extract was recorded in Table 6.

Preparation of aqueous extract

200 gm of air dried and finely ground plant material was extracted with distilled water by cold maceration in a round bottom flask for 24 hrs. After 24 hrs, it was filtered through Whatman filter paper and the extract was collected in a round bottom flask. This process was repeated using marc for another two times and all the three filtrates were collected and combined. The solvent was removed by distillation and concentrated *in vacuo* in a rotary evaporator. The dried extract was placed in a desiccator and used for further studies. The colour, consistency and the percentage yield of the extract was recorded in Table 6.

Qualitative chemical tests

The 50% ethanol extract, 90% ethanol extract and aqueous extract were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, steroids, terpenoids, anthraquinone glycosides, flavonoids, tannins and phenolic compounds, carbohydrates, proteins and amino acids and mucilages[58-61]. The following tests were carried out to identify the various phytoconstituents present in 50% ethanol extract, 90% ethanol extract and aqueous extract.

1. Test for Carbohydrates

Molisch's test: To 2-3 ml of extract, added few drops of 95 % α-napthol solution in alcohol, shake and added conc. H_2SO_4 from sides of the test tube. Violet ring was formed at junction of two layers.

Fehling's solution test: Mixed 1 ml. Fehling's A and Fehling's B solutions, boiled for one minute. To this equal volume of extracts were added. The heated in boiling water bath for 5-10 min. First a yellow, then brick red precipitates was observed.

Benedict's solution test: Mixed equal volume of Benedict's reagent and extract in test tube. Heated in boiling water bath for 5 min. Solution was appeared red.

2. Test for Proteins and amino acids

Biuret test: To 3 ml of aqueous extract added 4% NaOH and few drops of 1% $CuSO_4$ solution. Violet or pink colour was appeared.

Test with Millon's reagent: Mixed 3 ml of extract with 5 ml Millon's reagent. White precipitates were not formed.

Xanthoproteic test: Mixed 3ml of extract with 1ml conc.H_2SO_4. White precipitates were formed. Precipitates turned to yellow when heated. Added NH_4OH, orange precipitates were not formed.

Ninhydrin solution test: Heated 3 ml of extract and 3 drops of 5% Ninhydrin solution in boiling water bath for 10 min. Purple or bluish colour was appeared.

3. Test for Flavonoids

Shinoda's test: To 2ml of extract added 2ml ethanol, few drops of conc. HCl and little amount of magnesium turnings. Pink colour was observed.

Lead acetate solution test: To small quantity of extract added lead acetate solution. Yellow coloured precipitates were formed.

Sodium hydroxide solution test: To small quantity of residue added increasing amount of Sodium hydroxide solution. Yellow colouration was observed.

4. Test for Tannins and Phenolic compounds

To 2-3 ml alcoholic extract added few drops of following reagents:

5% Ferric chloride solution: Deep blue-black colour was observed.

Lead acetate solution: White precipitates were formed.

Gelatin solution: White precipitates were formed.

Potassium Dichromate: Red precipitates were formed.

Acetic acid solution: Red coloured solution was formed.

Dilute Iodine solution: Transient red colour was observed.

Dilute Nitric acid: Reddish to yellow colour.

5. Test for Steroids

Liebermann-Burchard Reaction: Mixed 2 ml of extract with chloroform. To this 1-2 ml of acetic anhydride and 2 drops of Conc. sulphuric acid were added from sides of test tube. First red, then blue and finally green colour was appeared.

Salkowiski Reaction: To 2 ml of extract added, 2 ml chloroform, and 2ml Conc Sulphuric acid. Shake well, chloroform layer appeared red and acid layer showed greenish yellow fluorescence.

6. Test for Anthraquinone glycosides

Borntrager's test: To 3 ml of extract, added dil. H_2SO_4. Boiled and filtered. To the filtrate, equal volume of chloroform was added. Shake well and separated

chloroform layer. Ammonia was added into organic layer. No red or pink colour was formed.

7. Test for Alkaloids

Extract was dried and treated with few drops of dilute HCl. Filtered and subjected the filterate to no of tests.

Test with Dragendorff's reagent: To 2-3 ml of filtrate was added, few drops of Dragendorff's reagent. No orange brown precipitates were formed.

Test with Mayer's reagent: To 2-3 ml filtrate, added few drops of Mayer's reagent, cream coloured precipitates were not formed.

Test with Hager's reagent: To 2-3 ml filtrate, added few drops of Hager's reagent. Yellow colour precipitates were not formed.

Test with Wagner's reagent: To 2-3 ml filtrate added few drops of Wagner's reagent. Reddish brown precipitates were not formed.

8. Test for Triterpenoids

Test with Tin and Thionyl chloride: To 2-3 ml of extract, added 2 ml of Tin and Thionyl chloride. Pink colour was not observed.

9. Test for Mucilages

Alcoholic precipitation test: Added about 2 ml of extract slowly to alcohol with constant stirring. Filtered the precipitates and dried in air. Precipitates thus obtained were examined for the presence of carbohydrates.

The results of qualitative chemical tests are tabulated in Table 7.

Pharmacological Studies

Antidiabetic studies of *Coldenia procumbens*

Animals

The experimental protocol used in the present study was approved by Institutional Animal Ethical Committee (IAEC). Male Wister rats weighing 160-180g and 3-4 months old were selected for study. These rats were kept in 12h-12h dark/light cycle, temperature of 22 ± 2 ˚C, humidity of 50-60% maintained at Animal House. During the whole experimental period, animals were fed with a balanced commercial pellet diet. The animals before experiment were fed with water *ad libitum.*

Biochemical parameters

A number of factors had influenced the outcome of biochemical observations and control of these factors was important in assessment of the significance of any changes that were encountered. In particular regularity and accuracy of dosing, the close control of conditions in animal house, and the control of inter current disease all bear on the significance of biochemical results and need constant care.

Glucose Estimation

Glucose tests were performed for the diagnosis of diabetes mellitus. In diabetes mellitus, high blood glucose levels up to 500 mg/dl or more were encountered, depending on the severity of condition. Except in diabetes mellitus fasting glucose levels rarely exceeds 110 mg/dl. The collected serum samples of different study groups were subjected to serum glucose level estimation by enzymatic GOD-POD method by using glucose diagnostic kit.

Principle of GOD-POD Method

Glucose is oxidized by glucose oxidase into gluconic acid and hydrogen peroxide. Hydrogen peroxide in presence of peroxidase (POD) oxidizes the chromogen 4-aminoantipyridine / phenolic compound to a red coloured compound. The intensity of red coloured compound is proportional to the glucose concentration and was measured at 505nm.[62-64] The final colour is stable for 2 hours.

$$\text{Glucose} + O_2 \xrightarrow{\textbf{GOD}} \text{Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{Phenolic Compound} + \text{4-Amino-antipyrine} \xrightarrow{\textbf{POD}} \text{Red Compound} + 2H_2O$$

Reagents

Reagent 1: (Buffer/Enzymes/Chromogen)

Phosphate Buffer	95 mmol/L
4-aminoantipyrine	0.2 mmol/L
p- hydroxy Benzoic acid	5.9 mmol/L
Glucose Oxidase	>5000 U/L
Peroxidase	>50000 U/L

Standard Glucose (100 mg/dl)

Glucose	1 g/l

Reagent Reconstitution

One tablet was gently dissolved in 20 ml of distilled water in a clean beaker with continuous stirring. The solution was then transferred into a dark bottle and labeled "Working solution".

Procedure

The samples and the reconstituting reagent had brought to room temperature prior to use. The following general parameters were used and reagents prepared were given in Table 3.

General system parameters

Reaction type : End point

Reaction Slope : Increasing

Wavelength : 505 nm

Flowcell Temp : 30ºC

Incubation : 15 min at 37 ºC or 30 min at room temperature.

Sample Volume : 10 µl

Reagent Volume : 1 ml

Stdandard concentration: 100mg/dl

Zero setting with : Reagent Blank

The samples were incubated for 15 min at 37 ºC. Mixed and read.

Table 3. Reagents prepared for measuring glucose levels

Reagents used	Blank	Standard	Test
Working Solution	1ml	1ml	1ml
Standard	-	10 ml	-
Sample	-	-	10 µl

Total Cholesterol Estimation

The determination of serum cholesterol levels is considered to be significant in coronary artery disease, diabetes mellitus and various other diseases. The determination of serum cholesterol concentration is one of the most widely performed assays in biochemistry. Elevated serum cholesterol is supposed to be a risk factor in the development of arteriosclerosis and myocardial infarction. Cholesterol in blood is predominantly esterified with fatty acids and associated with lipoproteins. Total

cholesterol, i.e. the sum of free and esterified cholesterol, can be accurately and conveniently measured enzymatically using cholesterol oxidase and cholesterol esterase. Cholesterol esterase which has a broad specificity towards the various fatty acid residues is used to cleave cholesterol esters to free cholesterol. Subsequently, Cholesterol oxidase transforms the steroid alcohol into cholest-4-ene-3-one andhydrogen peroxide which can be quantitated by a spectrophotometric assay e.g. the oxidative coupling of 4-aminoantipyrine and phenol in the presence of peroxidase to form a chromogen[65-67].

Principle

Cholesterol Ester + H_2O_2 $\xrightarrow{\textbf{cholesterol esterase}}$ Cholesterol + Fatty Acids

Cholesterol + O_2 $\xrightarrow{\textbf{cholesterol oxidase}}$ Cholestenone + H_2O_2

$2H_2O_2$ + Phenol + 4- Aminoantipyrine $\xrightarrow{\textbf{peroxidase}}$ Red quinone + $4H_20_2$

The concentration of cholesterol in the sample was directly proportional to the intensity of the red complex which was measured at 500 nm.

One of the major uses of cholesterol was the synthesis of bile acids. These were synthesized in the liver from cholesterol and are secreted in the bile. They were essential for the absorption of fat from the contents of the intestine.

Reagents

Reagent 1 (Enzymes/chromogen)

Cholesterol Esterase	> 200 U/L
Cholesterol Oxidase	> 250 U/L
Peroxidase	>1000 U/L
4-amino antipyrine	0.5 mmol/L
Reagent 1 A (Buffer)	
Pipes buffer, pH 6.90	50mmol/l
Phenol	24 mmol/l
Sodium cholate	0.5 mmol/l
Standard (Cholesterol 200mg/dl)	

Procedure

The samples and the reconstituent reagent should be brought to room temperature prior to use. The following general parameters were used and reagents prepared given in Table 4.

General System Parameters

Reaction type : End point

Reaction Slope : Increasing

Wavelength : 500 nm

Flowcell Temp : 30ºC

Incubation : 5 min at 37 ºC or 15 min at room temperature.

Sample Volume : 10 µl

Reagent Volume. : 1 ml

Standard concentration: 200mg/dl

Zero setting with : Reagent Blank

Set the instrument using above parameters

The samples were incubated for 15 min at 37 ºC. Mixed and read.

Table 4. Reagents prepared for measuring total cholesterol levels

Reagent used	Blank	Standard	Test
Reconstituting Reagent	1 ml	1 ml	1 ml
Standard	-	10 µl	-
Sample	-	-	10 µl

Triglycerides

Triglycerides are the chemical form in which most fat exists in food as well as in the body. They are also present in blood plasma and, in association with hyperlipoproteinimia, diabetes mellitus etc. Lipase hydrolyses triglycerides sequentially to di & monoglycerides and finally to glycerol. Glycerol Kinase (GK) using ATP as PO_4 source converts Glycerol liberated to Glycerol-3-Phosphate (G-3-Phosphate). G-3-Phosphate Oxidase (GPO) oxidises G-3-Phosphate & forms dihydroxy acetone phosphate and hydrogen peroxide. Peroxidase (POD) uses the hydrogen peroxide formed, to oxidise 4-Aminoantipyrine and TOOS (N-ethyl-N-sulphohydroxy propyl-m Toluidine) to a purple coloured complex. The absorbance of the coloured complex is measured at 546 nm (530-570 nm or with yellow filter) which is proportional to Triglyceride concentration.[68,69]

Principle

$$\text{Triglycerides} + H_2O_2 \xrightarrow{\textit{lipoprotein lipase}} \text{Glycerol} + \text{Fatty Acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\textbf{\textit{Glycerol kinase}}} \text{Glycerol-3-Phospate} + \text{ADP}$$

Glycerol-3 Phospate + O_2 $\xrightarrow{\text{GPO}}$ Dehydroxyacetone Phospate + H_2O_2

$2H_2O_2$+4 Aminoantipyrine + ADPS $\xrightarrow{\textit{peroxidase}}$ Red quinone + $4H_2O_2$

GPO = Glycerol- 3-Phospate Oxidase

ADPS = N-Ethyl N-Sulphopropyl–n-anisidine

The intensity of purple coloured complex formed during the reaction was directly proportional to the triglycerides concentration in the sample measured at 546 nm. Triglycerides in plasma were derived from fats eaten in foods or made in the body from other energy sources like carbohydrates. Calories ingested in a meal and not used immediately by tissues were converted to triglycerides and transported to fat cells to be stored. Hormones regulate the release of triglycerides from fat tissue so that they meet the body's needs for energy between meals.

Reagents (Enzymes/chromogen)

Lipoprotein lipase	> 1100 U/L
Glycerol kinase	> 800 U/L
Glycerol 3-Phosphate Oxidase	>5000 U/L
Peroxidase	>350 U/L
4-Aminoantipyrine	0.7 mmol/l
ATP	0.3mmol/l

Reagent 1 A (Buffer)

Pipes buffer, pH 7.50	50mmol/l
ADPS	1 mmol/l
Magnesium salt	15 mmol/l

Standard (Triglycerides 200mg/dl)

Glycerol (Trig. Equivalent)	2 g/l

Procedure

The samples and the reconstituting reagent were brought to room temperature prior to use. The following general parameters were used and reagents prepared were given in Table 5.

General System Parameters

Reaction type	: End point
Reaction Slope	: Increasing
Wavelength	: 546 nm
Flowcell Temp	: 30ºC

Incubation : 5 min at 37 ºC

Sample Volume : 10 µl

Reagent Volume : 1 ml

Standard concentration : 200 mg/dl

Zero setting With : Reagent Blank

Set the instrument using above parameters

Table 5. Reagents used for measuring Triglycerides levels

Reagents used	Blank	Standard	Test
Reconstituting Reagent	1 ml	1 ml	1 ml
Standard	-	10 µl	-
Sample	-	-	10 µl

Experimental Models

Fasting glucose model

Male Wistar rats weighing 160-180g, 3-4 months old were used for study and maintained at temperature of 22 ± 2˚C and humidity of 50-60% with water *ad libitum* during the whole experimental period in animal house.

Each group consists of 6 animals. Extracts were suspended in 1% w/v gum acacia and administered orally. Blood glucose levels were estimated at 0 h and 3 h after test extract administration.

Experimental protocol

Rats were divided into 4 groups of 6 rats in each group as mentioned below:

Group I: Rats were treated with vehicle (vehicle control)

Group II: Rats were treated with 90% ethanol extract (250 mg/kg p.o.)

Group III: Rats were treated with 50% ethanol extract (250 mg/kg p.o.)

Group IV: Rats were treated with aqueous extract (250 mg/kg p.o.).

Oral Glucose Tolerance test

In this test, 90% ethanol extract and 50% ethanol extract at a dose of 250 mg/kg p.o., were used for antidiabetic evaluation. Aqueous extract was not used. Male Wistar rats weighing 160-180 g and 3 to 4 months old were used for study. The

animals were maintained at temperature of 22 ± 2˚C and humidity of 50-60% with water *ad libitum* during the whole experimental period in animal house.

Each group consists of 6 animals. Extracts were suspended in 1% w/v gum acacia and administered orally. Glucose Solution was prepared in concentration of 1.5g/10 ml and administered one and half hour after drug administration. Blood glucose levels were measured at 0 min, 30 min, 60 min and 90 min after glucose administration.

Experimental protocol

Rats were divided into 4 groups of 6 rats in each group as given below

Group I: Rats were treated with vehicle (control).

Group II: Rats were treated with 90% ethanol extract (250 mg/kg p.o.)

Group III: Rats were treated with 50% ethanol extract (250 mg/kg p.o.)

Group IV: Rats were treated with glibenclamide (0.5 mg/kg p.o.)

Streptozotocin induced diabetes Model

Streptozotocin was used in dose of 45 mg/kg i.p. for the induction of diabetes. Rats with blood glucose levels more than 250 mg/dl and less than 400 mg/dl were selected for study. Male Wistar rats weighing 160-180g, 3-4 months old were used for study and maintained at 22 ± 2˚C and humidity of 50-60% with water *ad libitum* during the whole experimental period in animal house.

In this model, 90% ethanol extract and 50% ethanol extract at a dose of 250mg/kg p.o., were used for antidiabetic evaluation. Aqueous extract was not used. Each group consists of 6 animals. Extracts were suspended in 1%w/v gum acacia and administered orally daily for 21 days to rats. Blood glucose levels were estimated before induction of diabetes and 1 week after STZ administration. Rats with blood glucose levels more than 250 mg/dl but less than 400mg/dl were selected for further study.

Experimental protocol

Rats were divided into 4 groups of 6 rats in each group as given below

Group I: No treatment was given throughout the protocol (normal control)

Group II: No treatment was given throughout the protocol (STZ control)

Group III: Rats were treated with 90% ethanol extract (250 mg/kg p.o.)

Group IV: Rats were treated with 50% ethanol extract (250 mg/kg p.o.)

Statistical Analysis

The results were expressed as mean ± S.E.M. The data was analyzed using one- way analysis of variance (ANOVA) followed by Tukey's Multiple comparison test. The P value of less than 0.05 was considered to be statistically significant.

5. RESULTS AND DISCUSSION

Extraction

Preparation of Extracts

The Aqueous extract prepared was blackish brown powder with a percentage yield of 12.2%w/w. The 50% ethanol extract prepared was blackish brown in colour with a percentage yield of 8.4 % w/w and 90% ethanol extract was blackish brown in colour with % yield of 7.1%w/w. The results are summarized in Table 6. Aqueous extract showed the highest percentage of yield and 90% ethanol extract showed lowest percentage of yield among these three extracts prepared from the leaves of *Coldenia procumbens*.

Table 6. Colour, consistency and % yield of extracts

S.no	Extract	Colour	Consistency	% yield (w/w)
1.	Aqueous extract	Blackish brown	Solid mass	12.2%
2.	50% ethanol extract	Blackish brown	Solid mass	8.4 %
3.	90% ethanol extract	Blackish brown	Solid mass	7.1 %

Qualitative chemical tests

The 90% ethanol extract, 50% ethanol extract and aqueous extracts of *Coldenia procumbens* were subjected for the phytochemical screening. The qualitative chemical tests revealed the presence of tannins and phenolic compounds, flavonoids, carbohydrates, amino acids and steroids in 50% ethanol extract and 90% ethanol extract. Aqueous extract showed the presence of tannins and phenolic compounds, flavonoids, carbohydrates, proteins, amino acids and mucilages. Alkaloids are absent in all the three extracts. Results of the qualitative chemical test are shown in Table 7.

Table 7. Qualitative phytochemical analysis

SI. No.	Tests	Aqueous extract	90% Ethanol extract	90% Ethanol extract
1.	Alkaloid	-	-	-
2.	Carbohydrates	+	+	+
3.	Proteins	+	-	-
4.	Amino Acids	+	+	+
5.	Steroids and Sterols	-	+	+
6.	Anthraquinones	-	-	-
7.	Flavonoids	+	+	+
8.	Tannins and Phenol compounds	+	+	+
9.	Triterpenoids	-	-	-
10.	Saponins	-	-	-
11.	Fixed oils	-	-	-
12.	Gums an Mucilage	-	-	-

Antidiabetic evaluation of *Coldenia procumbens*

Antidiabetic evaluation of *Coldenia procumbens* had been done by using three models, fasting glucose model, oral glucose tolerance test and streptozotocin induced diabetes model. The oral glucose tolerance test (OGTT) is a widely used procedure that was originally developed to classify carbohydrate tolerance[70]. However, because plasma glucose and insulin responses during this test reflect the ability of pancreatic -cells to secrete insulin and the sensitivity of tissues to insulin the OGTT has also been often used to evaluate -cell function and insulin resistance.[71,72]

Diabetes is induced by streptozotocin (STZ), a glucosamine-nitrosourea compound derived from *Streptomyces achromogenes* that is used clinically as a chemotherapeutic agent in the treatment of pancreatic β cell carcinoma. STZ damages pancreatic β cells, resulting in hypoinsulinemia and hyperglycemia[73]. STZ can induce a diabetic state in 2 ways, depending on the dose. The selectivity for β cells is associated with preferential accumulation of the chemical in β cells after entry through the GLUT2 glucose transporter receptor: chemical structural similarity with glucose allows STZ to bind to this receptor. The mode of action has best been demonstrated in mouse studies. At high doses, typically given singly, STZ targets β cells by its alkylating property corresponding to that of cytotoxic nitrosourea compounds[74]. At low doses, generally given in multiple exposures, STZ elicits an immune and inflammatory reaction, presumably related with the release of glutamic acid decarboxylase autoantigens. Under this condition, the destruction of β cells and induction of the hyperglycemic state is associated with inflammatory infiltrates including lymphocytes in the pancreatic islets[75]. STZ has well-known adverse side effects, which include hepatotoxicity and nephrotoxicity[76-78].

Fasting glucose model

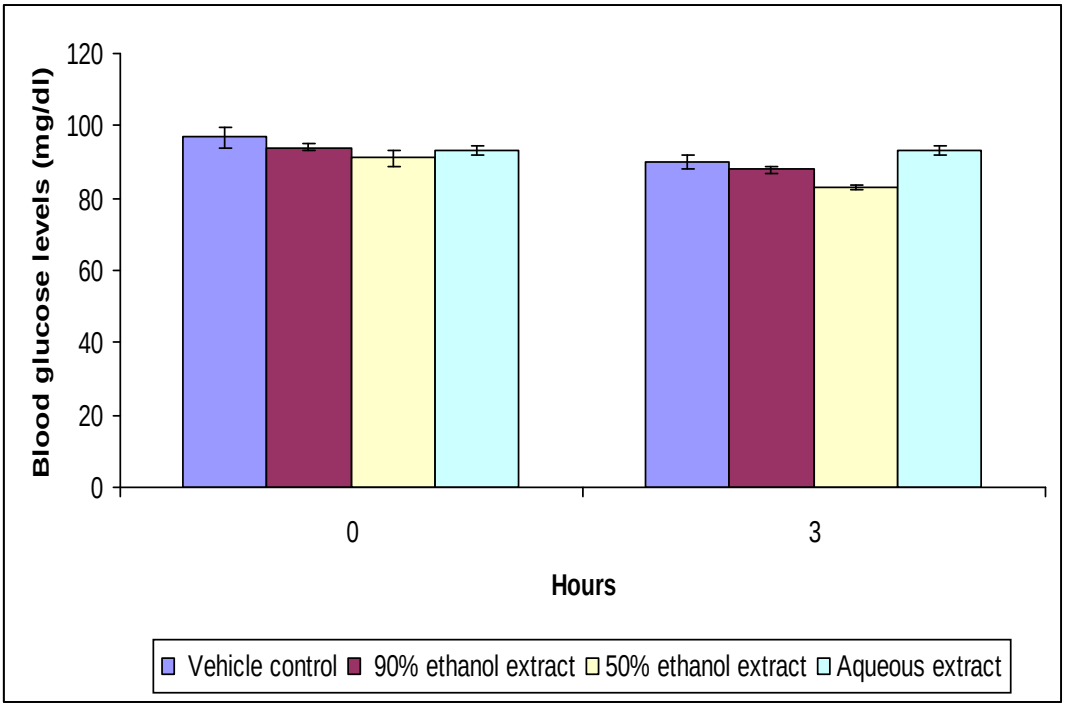
Rats in group I (vehicle control) showed glucose level of 96.6 ± 2.89 at 0 hour and 90.20 ± 2.12 at 3 hours respectively. In group II, the rats administered with 90% ethanol extract showed glucose level of 94.00 ± 1.09 at 0 hour and 88.20 ± 1.00 at 3 hours respectively. Similarly, in group III and group IV rats administered with 50% ethanol extract and aqueous extract did not reduce blood glucose level in rats. These results indicate that all the three extracts at a dose of 250mg/kg do not produce any hypoglycaemic activity in normal rats. Results are given in Table 8 and Figure 13.

Table 8. Effect of *Coldenia procumbens* extracts on blood glucose level in fasting glucose model

Experimental Groups	Blood glucose level (Mean ± S.E.) (mg/dl)	
	0 h	3 h
Group I - Vehicle control	96.60 ± 2.89	90.20 ± 2.12
Group II -90% Ethanol Extract (250 mg/kg)	94.00 ± 1.09	88.20 ± 1.00
Group III -50% Ethanol extract (250 mg/kg)	90.60 ± 2.39	82.80 ± 0.79
Group IV- Aqueous extract (250 mg/kg)	92.60 ± 1.21	93.80 ± 1.37

Results were expressed as mean ± S.E.M

Fig. 13. Effect of *Coldenia proumbens* on blood glucose levels in fasting glucose model



Results were expressed as Mean±S.E.M.

Oral glucose tolerance test (OGTT)

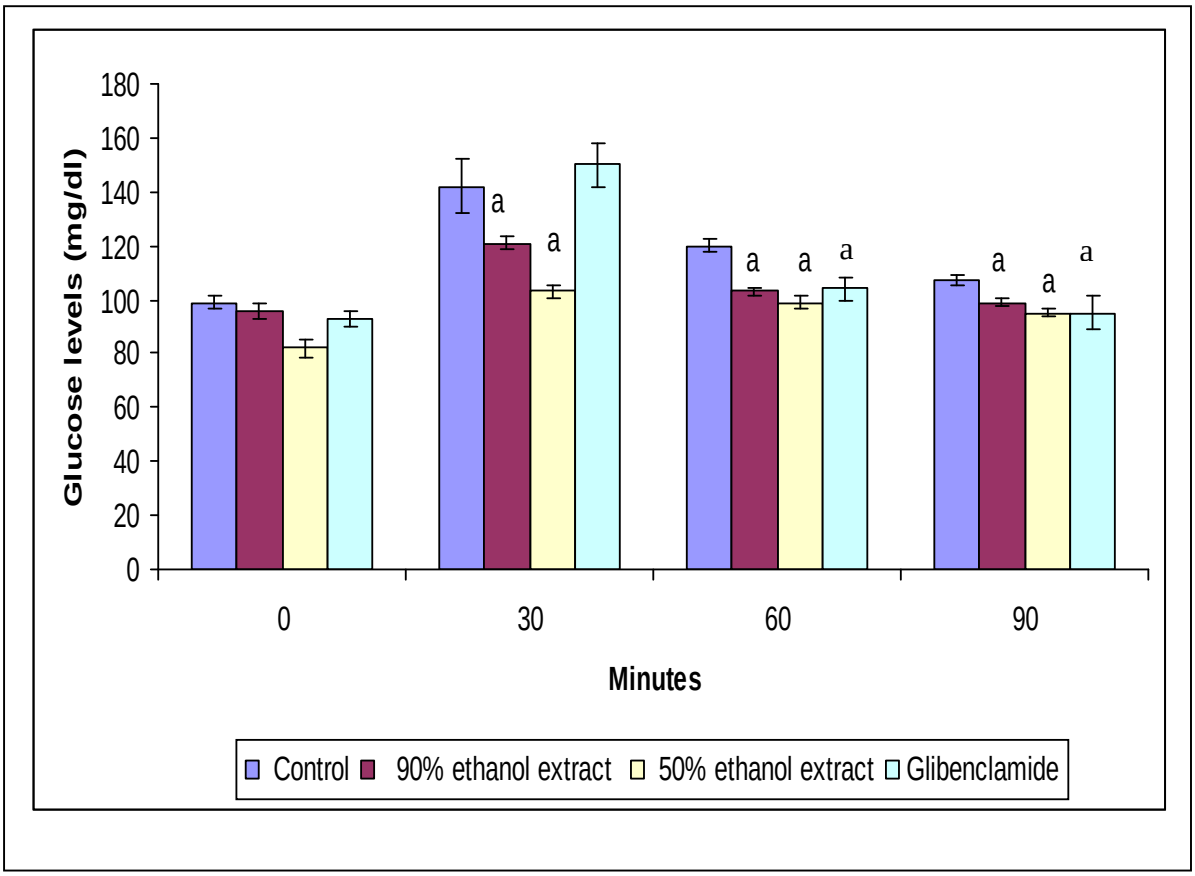
Rats in group I (control group) showed blood glucose levels of 99.2 ± 2.75 at 0 hour, 142.00 ± 9.77 at 30 minutes, 119.80 ± 2.33 at 60 minutes and 107.00 ± 1.89 at 90 minutes. In this control group, there is increase in blood glucose levels at 30, 60 and 90 minutes. Maximum increase in blood glucose levels was observed at 30 minutes after glucose solution administration. In group II, III, and IV the blood glucose levels were 96.20 ± 2.94, 81.80 ± 3.05 and 2.25 ± 2.95 respectively at 0 hour. After 30 minutes of glucose solution administration to rats of group II, III and IV, it showed decrease in blood glucose levels when compared to group I. 90% ethanol extract treated group (group II) showed reduction in blood glucose levels at 30, 60 and 90 minutes when compared to group I. The rats of group III, that received 50% ethanol extract at dose of 250 mg/kg also showed the reduction in blood glucose levels at 30, 60 and 90 minutes. The standard drug (glibenclamide-0.5mg/kg) treated group of rats showed the reduction of blood glucose level at 60 and 90 minutes. In all the cases, the extract and standard drug treated animals showed near normal level of blood glucose at 90 minutes. The 90% ethanol extract treated group showed more reduction of blood glucose level at 60 minutes whereas 50% ethanol extract treated group showed significant blood glucose level reduction at 30 minutes. The standard drug (glibenclamide-0.5mg/kg) reduced blood glucose level significantly at 60 minutes whereas it did not produce any reduction in blood glucose level at 30 minutes. Based on these results, it is evident that extracts at a dose of 250 mg/kg body weight and standard drug (glibenclamide-0.5mg/kg) showed antihyperglycaemic activity in oral glucose tolerance test. Results are given in Table 9 and Figure 14.

Table 9: Effect of *Coldenia procumbens* extracts on blood glucose level in Oral glucose tolerance test

Experimental Groups	Blood glucose level (Mean ± S.E.) (mg/dl)			
	0 min.	30min.#	60 min.#	90 min.#
Group I – Control group	99.20±2.75	142.00±9.77	119.80±2.33	107.00±1.89
Group II – 90% Ethanol extract (250mg/kg)	96.20±2.94	121.00±2.19[a]	103.00±1.71[a]	99.00±1.51[a]
Group III – 50% Ethanol extract (250 mg/kg)	81.80±3.05	103.40±2.26[a]	99.00±2.05[a]	95.20±1.57[a]
Group IV- Glibenclamide (0.5 mg/kg)	92.80±2.95	137.70±3.64	104.00±4.45[a]	95.20±6.11[a]

Results were expressed as mean ± S.E.M. # Timing post glucose administration
'a' = p<0.05 compared to control

Fig 14. Effect of *Coldenia procumbens* extracts on blood glucose levels in Oral glucose tolerance rats



Results were expressed as mean ± S.E.M. 'a'= p<.05 compared to vehicle control

The glucose tolerance test is the most widely used test in rodents to determine whether a genetically engineered (e.g., transgenic or knockout) or dietary-induced mouse is glucose intolerant and diabetic. Variables such as fasting duration, route, and amount of glucose administration, as well as state of consciousness, may have a large impact on the measured glucose tolerance in mice. Fasting duration has a major impact on both the plasma glucose and insulin levels. The overnight- and 24-h-fasted mice has suppressed basal plasma glucose and insulin levels[79,80].

In epidemiological studies, for example, fasting plasma insulin concentrations have been used as an index of insulin resistance, and the 30 -min ratio of changes in plasma insulin and glucose have been used as an index of -cell function[81,82].

In the present study, the 90% ethanol extract treated group showed more reduction of blood glucose level at 60 minutes whereas 50% ethanol extract treated group showed significant blood glucose level reduction at 30 minutes. The standard drug glibenclamide reduced blood glucose level significantly at 60 minutes. So it may be concludes that 50% ethanol extract show better antidiabetic activity than 50% ethanol extract.

Streptozotocin induced diabetes model

Rats in group I (normal control) showed blood glucose levels of 88.30 ± 3.20, 89.10 ± 4.01, 84.80 ± 5.51, 86.90 ± 4.31 and 85.90 ± 2.90 at 0, 7, 14, 21 and 28 day of experiment. In this normal control group, no change in blood glucose levels is observed at 7, 14, 21 and 28 day of experiment. In group II, (Streptozotocin control) rats showed blood glucose levels of 338.50 ± 11.73, 349.80 ± 21.90, 342.00 ± 20.27 and 300.10 ± 23.03 at 7, 14, 21 and 28 day of experiment. This group showed significant increase in blood glucose levels at 7, 14, 21, and 28 day of experiment compared to group I. Nearly 4 times increase in blood glucose level was observed in Streptozotocin control animals. Rats in group III, showed blood glucose levels of 333.60 ± 14.02, 233.40 ± 17.30, 246.70 ± 10.35 and 191.95 ± 12.60 at 7, 14, 21 and 28 day of experiment respectively. In this group, significant reduction in blood glucose levels were observed at 14, 21, and 28 day of experiment when compared to group II. Rats in group IV showed blood glucose levels of 340.01 ± 10.53, 175.05 ± 14.30, 162.80 ± 7.15, and 146.85 ± 14.18 at 7, 14, 21, and 28 day of experiment

respectively. In this group, significant reduction in blood levels was observed at 14, 21, and 28 day of experiment when compared to group II.

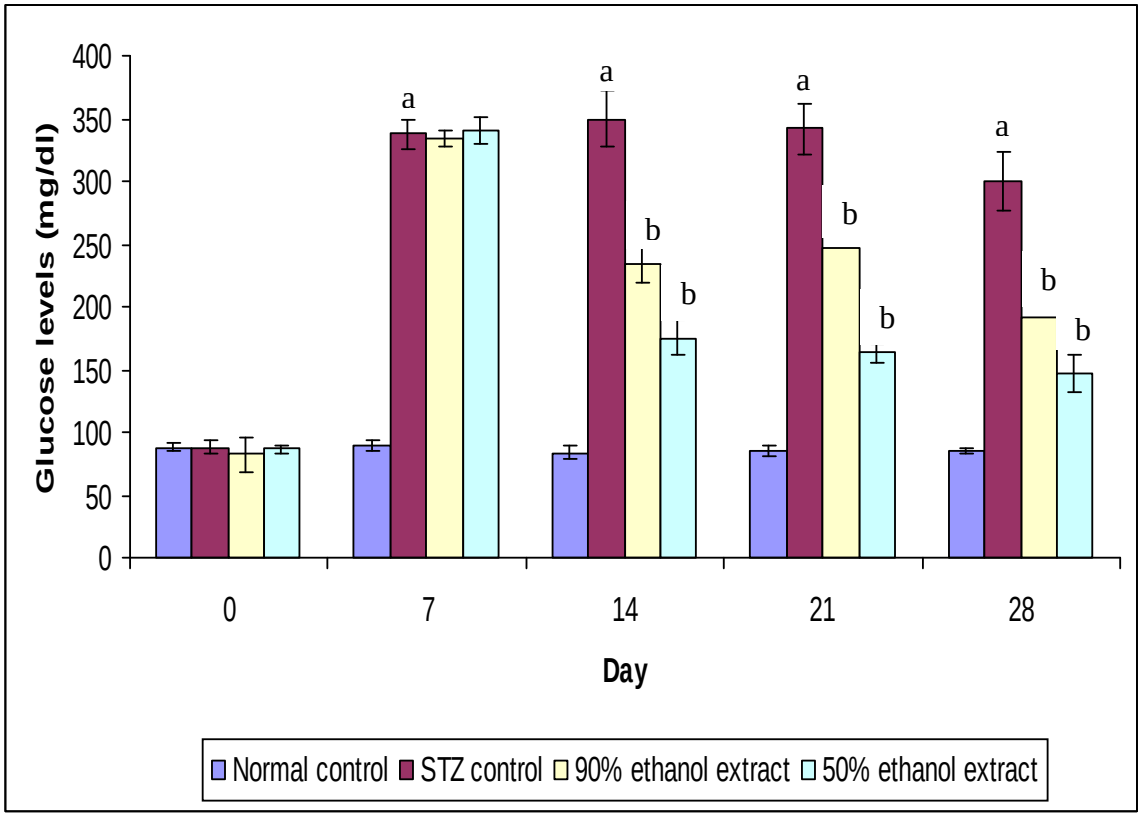
Extracts treated group of rats showed significant reduction of blood glucose levels at 14, 21 and 28 day indicating the antidiabetic activity of the extract. When compared to 90% ethanol extract, higher reduction of blood glucose levels were observed in 50% ethanol extract treated group at day 14, 21 and 28. Based on these results, it is evident that both the extracts at a dose of 250 mg/kg body weight showed antidiabetic activity in streptozotocin induced diabetes model. However, higher reduction of blood glucose levels was observed in rats treated with 50% ethanol extract treated group. Results are given in Table 10 and Figure 15.

Table 10: Effect of *Coldenia procumbens* extracts on blood glucose levels in streptozotocin induced diabetes model

Experimental Groups	Blood glucose level (Mean ± S.E.) (mg/dl)				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Group I- Normal control	88.30 ± 3.20	89.10 ± 4.01	84.80 ± 5.51	86.90 ± 4.31	85.90 ± 2.90
Group II- STZ control	87.60 ± 5.96	338.50 ± 11.73[a]	349.80 ± 21.90[a]	342 ± 20.27[a]	300.10 ± 23.03[a]
Group III- 90% Ethanol Extract (250 mg/kg)	82.10 ± 4.47	333.60 ± 14.02	233.40 ± 17.30[b]	246.70 ± 10.35[b]	191.95 ± 12.60[b]
Group IV- 50% Ethanol extract (250 mg/kg)	86.90 ± 3.32	340.01 ± 10.53	175.05 ± 14.30[b]	162.80 ± 7.15[b]	146.85 ± 14.18[b]

Results were expressed as mean ± S.E.M. 'a' = $p < 0.05$ compared to Normal control
'b' = $p < 0.05$ compared to STZ control

Fig 15. Effect of *Coldenia procumbens* extracts on blood glucose levels in streptozotocin induced diabetes model



Results were expressed as Mean ± S.E.M. 'a' = $p < 0.05$ compared to Normal ontrol; 'b' = $p < 0.05$ compared to STZ control

Streptozotocin induced diabetes is a well-documented model of experimental diabetes. Previous reported literature indicates that the type of diabetes and characteristics differ with the employed dose of STZ and animal and species used[83-86].

Streptozotocin induced diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia. STZ is a pancreatic cell toxin that induces rapid and irreversible necrosis of cells. Whereas a single diabetogenic dose of STZ (70-250 mg/kg, body weight) has been demonstrated to induce complete destruction of cells in most species within 24 hour, multiple sub-diabetogenic doses of STZ partially damage islets, thereby triggering an inflammatory process leading to macrophage and subsequent lymphocyte infiltration, which is followed by the onset of insulin deficiency[87].

Effect of *Coldenia procumbens* extracts on total cholesterol levels in streptozotocin induced diabetes model

Rats in group I (normal control) showed total cholesterol levels of 57.07 ± 4.40, 60.11 ± 4.87, 59.98 ± 4.30 at 0, 14 and 28 day of experiment respectively. In this normal control group, no change in total cholesterol levels were obtained at 14, and 28 day of experiment. Rats in group II showed total cholesterol levels of 61.09 ± 0.98, 57.75 ± 1.41 and 58.12 ± 4.36 at 0, 14 and 28 days of experiment respectively. Rats in group III, showed total cholesterol levels of 60.18 ± 4.31, 56.33 ± 0.94 and 59.64 ± 3.57 at 0, 14, and 28 days of experiment respectively. Rats in group IV showed total cholesterol levels of 62.01 ± 3.80, 50.95 ± 1.41 and 57.29 ± 8.64 at 0, 14 and 28 days of experiment respectively. There was no significant changes in the total cholesterol levels in all group of rats. Results are given in Table 11 and Figure 16.

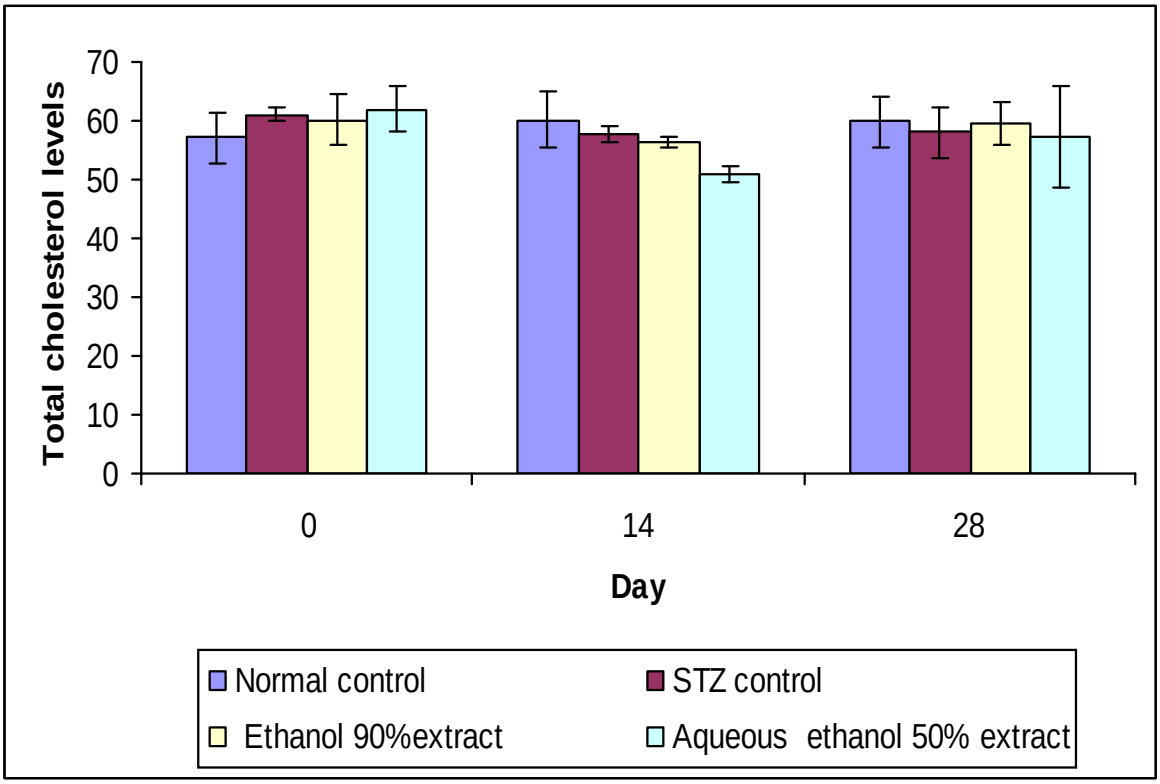
Diabetes tends to lower "good" cholesterol levels and raise triglyceride and "bad" cholesterol levels, which increases the risk for heart disease and stroke. This common condition is called diabetic dyslipidemia. Diabetic dyslipidemia means lipid profile is going in the wrong direction. It's a deadly combination that puts patients at risk for premature coronary heart disease and atherosclerosis — where the arteries become clogged with accumulated fat and other substances. Studies show a link between insulin resistance, which is a precursor to type 2 diabetes, and diabetic dyslipidemia, atherosclerosis and blood vessel disease. These conditions can develop even before diabetes is diagnosed. Hence, reduction in the cholesterol is always beneficial. However, the extracts used were not showed any effect on the cholesterol levels.

Table 11: Effect of *Coldenia procumbens* extracts on total cholesterol levels in streptozotocin induced diabetes model

Experimental Groups	Total Cholesterol levels (Mean ±S.E.) (mg/dl)		
	Day 0	Day 14	Day 28
Group I- Normal control	57.07±4.40	60.11±4.87	59.98±4.30
Group II- STZ control	61.09±0.98	57.75±1.41	58.12±4.36
Group III- 90% ethanol extract (250 mg/kg)	60.18±4.31	56.33±0.94	59.64±3.57
Group IV- 50% ethanol extract (250 mg/kg)	62.01±3.80	50.95±1.41	57.29±8.64

Results were expressed as Mean ± S.E.M.

Fig 16. Effect of *Coldenia procumbens* extracts on total cholesterol levels in streptozotocin induced diabetes model



Results were expressed as Mean ± S.E.M.

Effect of *Coldenia procumbens* extracts on triglyceride levels in streptozotocin induced diabetes model

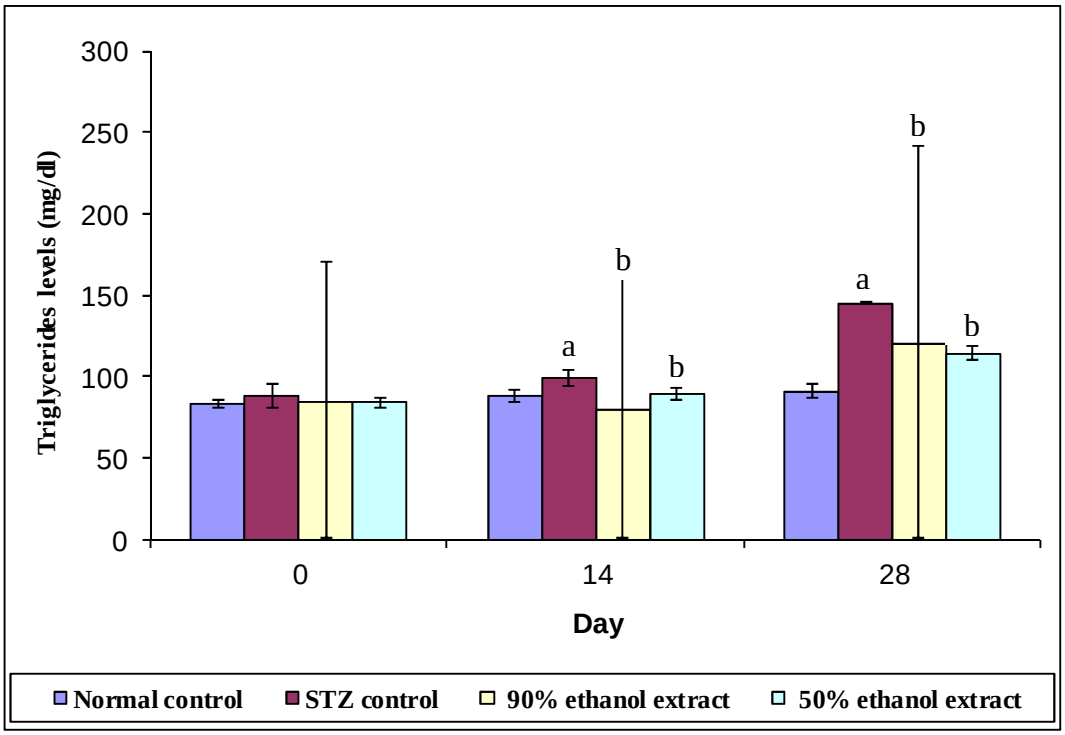
Rats in group I (normal control) showed triglyceride level of 83.10 ± 2.82, 87.90 ± 3.23 and 90.98 ± 3.88 at 0, 14 and 28 day of experiment respectively. In group I, no change in triglyceride level is observed at 14 and 28 day of experiment. Rats in group II, showed triglyceride level of 88.07 ± 7.11, 99.08 ± 5.01 and 145.00 ± 0.11 at 0, 14, and 28 day of experiment respectively. In group II, significant increase in triglyceride level is observed at 14 and 28 day of experiment compared to normal control. Rats in group III, showed triglyceride level of 84.67 ± 3.25, 79.80 ± 3.54 and 120.01 ± 3.91 at 0, 14 and 28 day of experiment respectively. In group III, significant reduction in triglyceride level was observed at 14 and 28 day of experiment, when compared to group II. Rats in group IV, showed triglyceride levels of 83.90 ± 4.51, 89.10 ± 2.76 and 114.30 ± 3.14 at 0, 14 and 28 day of experiment respectively. In group IV, significant reduction in triglyceride level was observed at 14 and 28 day of experiment compared to group II. Based on these results, it is evident that extracts at 250 mg/kg of body weight showed reduction in triglyceride levels. Results are given in Table 12 and Figure 17.

Table 12: Effect of *Coldenia procumbens* extracts on triglyceride levels in streptozotocin induced diabetes model.

Experimental Groups	Triglyceride levels (Mean ± S.E.) (mg/dl)		
	Day 0	Day 14	Day 28
Group I- Normal control	83.10 ± 2.82	87.90 ± 3.23	90.98 ± 3.88
Group II- STZ control	88.07 ± 7.11	99.08 ± 5.01[a]	145.00 ± 0.11[a]
Group III- 90% ethanol extract (250 mg/kg)	84.67 ± 3.25	79.80 ± 3.54[b]	120.01 ± 3.91[b]
Group IV- 50% ethanol extract (250 mg/kg)	83.90 ± 4.51	89.10 ± 2.76[b]	114.30 ± 3.14[b]

Results were expressed as Mean ± S.E.M. a' = $p<0.05$ compared to Normal control 'b'= $p<0.05$ compared to STZ control group.

Fig 17. Effect of *Coldenia procumbens* extracts on triglyceride levels in streptozotocin induced diabetes model



Results were expressed as Mean ± S.E.M. 'a' = $p<0.05$ compared to Normal control 'b'= $p<0.05$ compared to STZ control group**.**

People with diabetes tend to have high levels of triglycerides and LDL cholesterol, combined with low levels of HDL, the "good" cholesterol, a condition known as diabetic dyslipidemia. The greatest link between diabetes and triglycerides was found. It showed that diabetics with high triglyceride levels were much more likely to develop nerve damage, or neuropathy. People suffering from diabetic neuropathy experience a painful tingling and numbing in their extremities as nerves are damaged or lost. High triglyceride levels that accompany either normal or impaired fasting glucose predict the development of type 2 diabetes and therefore, hypertriglyceridemic states should prompt surveillance to rule out type 2 diabetes[88].

200 mg/dL168 associated with decreased HDL-C and small, dense LDL particles[89]. Hence, reduction in the triglyceride is necessary to avoid future event of cardiovascular diseases/complications. However, the extracts used showed significant reduction in the levels of triglycerides in the animals further confirms the antidiabetic activity.

6. SUMMARY AND CONCLUSION

The present study was designed to evaluate the antidiabetic potential of *Coldenia procumbens*. Preliminary chemical studies included qualitative chemical tests. Antidiabetic evaluation of *Coldenia procumbens* was done using 18 hr fasting glucose model, oral glucose tolerance test and streptozotocin induced diabetes model.

Qualitative chemical tests revealed the presence of carbohydrates, flavonoids, steroids, tannins and phenolic compounds in 50% ethanol extract and 90% ethanol extract and presence of carbohydrates, proteins and amino acids, flavonoids, steroids, tannins and phenolic compounds in aqueous extract.

Antidiabetic studies on 50% ethanol extract, 90% ethanol extract and aqueous extract of *Coldenia procumbens* was carried out using fasting glucose model, oral glucose tolerance test and streptozotocin induced diabetes model. 90% ethanol extract, 50% ethanol extract and aqueous extracts in dose of 250 mg/kg were devoid of hypoglycemic activity in fasting glucose model. 90% ethanol extract and 50% ethanol extract in the dose of 250 mg/kg showed antihyperglycaemic activity in oral glucose tolerance test. 50% ethanol extract is more effective than 90% ethanol extract in oral glucose tolerance test. 90% Ethanol extract and 50% Ethanol extract in dose of 250mg/kg showed antidiabetic activity in streptozotocin induced diabetic rats. 50% ethanol extract is more effective than 90% ethanol extract in the dose of 250 mg/kg in streptozotocin induced diabetic rats. 50% ethanol extract of *Coldenia procumbens* showed significant reduction in triglycerides levels in streptozotocin induced diabetic rats.

Effect on blood glucose level in fasting glucose model: In the single dose treatment at 250mg/kg p.o., none of the three extracts of *Coldenia procumbens* showed hypoglycemic activity in fasting glucose model which indicates that these extracts were devoid of insulin secreting or insulin like activity.

Effect on blood glucose level in oral glucose tolerance test: In oral glucose tolerance test (OGTT) the lowering of blood glucose was observed at 30 and 60min. post glucose administration when compared to control.

Effect on blood glucose level in streptozotocin induced diabetes model: In streptozotocin induced diabetes model, both the extracts i.e. 50% ethanol extract and 90% ethanol extract of *Coldenia procumbens* exhibited varied degree of reversal of

diabetic condition with maximum effect in 50% ethanol extract when compared to the streptozotocin control. In the group treated with 50% ethanol extract, the blood glucose level significantly reduced to 146.85 ± 14.18 mg/dl on day 28 as compared to that of 300.10 ± 23.03 mg/dl in streptozotocin control.

The 50% ethanol extract may be subjected fractionation in order to enrich an active fraction and thereby the phytochemical responsible for this antidiabetic activity may be identified, isolated and characterized. It may be concluded that the leaves of *Coldenia procumbens* possess antidabetic activity in animal models.

7. References

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